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Volume IV of IV

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Although this document is principally concerned with the health and welfare effects of lead, other scientific data are presented and evaluated in order to provide a better understanding of this pollutant in the environment. To this end, the document includes chapters that discuss: the chemistry and physics of the pollutant; analytical levels; atmospheric chemistry and dispersion modeling; effects on vegetation; and respiratory, physiological, toxicological, clinical and epidemiological aspects of human exposure.

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Environmental Criteria and Assessment Office
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The document evaluates and assesses scientific information on the health and welfare effects associated with exposure to various concentrations of lead in ambient air. The literature through 1985 has been reviewed thoroughly for information relevant to air quality criteria, although the document is not intended as a complete and detailed review of all literature pertaining to lead. An attempt has been made to identify the major discrepancies in our current knowledge and understanding of the effects of these pollutants.

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LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrometry
Ach	Acetylcholine
ACTH	Adrenocorticotrophic hormone
ADCC	Antibody-dependent cell-mediated cytotoxicity
ADP/O ratio	Adenosine diphosphate/oxygen ratio
AIDS	Acquired immune deficiency syndrome
AIHA	American Industrial Hygiene Association
AII	Angiotensin II
ALA	Aminolevulinic acid
ALA-D	Aminolevulinic acid dehydrase
ALA-S	Aminolevulinic acid synthetase
ALA-U	Aminolevulinic acid in urine
APDC	Ammonium pyrrolidine-dithiocarbamate
APHA	American Public Health Association
ASTM	Amercian Society for Testing and Materials
ASV	Anodic stripping voltammetry
ATP	Adenosine triphosphate
B-cells	Bone marrow-derived lymphocytes
Ba	Barium
BAL	British anti-Lewisite (dimercaprol)
BAP	benzo(a)pyrene
BSA	Bovine serum albumin
BUN	Blood serum urea nitrogen
BW	Body weight
C.V.	Coefficient of variation
CaBP	Calcium binding protein
CaEDTA	Calcium ethylenediaminetetraacetate
$CaNa_2EDTA$	Calcium disodium ethylenediaminetetraacetate
CBD	Central business district
Cd	Cadmium
CDC	Centers for Disease Control
CEC	Cation exchange capacity
CEH	Center for Environmental Health
CFR	reference method
CMP	Cytidine monophosphate
CNS	Central nervous system
CO	Carbon monoxide
COHb	Carboxyhemoglobin
CP-U	Urinary coproporphyrin
C_{pah}	plasma clearance of p-aminohippuric acid
Cu	Copper
D.F.	Degrees of freedom
DA	Dopamine
δ-ALA	delta-aminolevulinic acid
DCMU	[3-(3,4-dichlorophenyl)-1,1-dimethylurea
DPP	Differential pulse polarography
DNA	Deoxyribonucleic acid
DTH	Delayed-type hypersensitivity
EEC	European Economic Community
EEG	Electroencephalogram
EMC	Encephalomyocarditis
EP	Erythrocyte protoporphyrin

LIST OF ABBREVIATIONS (continued).

EPA	U.S. Environmental Protection Agency
FA	Fulvic acid
FDA	Food and Drug Administration
Fe	Iron
FEP	Free erythrocyte protoporphyrin
FY	Fiscal year
G.M.	Grand mean
G-6-PD	Glucose-6-phosphate dehydrogenase
GABA	Gamma-aminobutyric acid
GALT	Gut-associated lymphoid tissue
GC	Gas chromatography
GFR	Glomerular filtration rate
HA	Humic acid
Hg	Mercury
hi-vol	High-volume air sampler
HPLC	High-performance liquid chromatography
i.m.	Intramuscular (method of injection)
i.p.	Intraperitoneally (method of injection)
i.v.	Intravenously (method of injection)
IAA	Indol-3-ylacetic acid
IARC	International Agency for Research on Cancer
ICD	International classification of diseases
ICP	Inductively coupled plasma emission spectroscopy
IDMS	Isotope dilution mass spectrometry
IF	Interferon
ILE	Isotopic Lead Experiment (Italy)
IRPC	International Radiological Protection Commission
K	Potassium
LDH-X	Lactate dehydrogenase isoenzyme x
LC_{50}	Lethyl concentration (50 percent)
LD_{50}	Lethal dose (50 percent)
LH	Luteinizing hormone
LIPO	Laboratory Improvement Program Office
ln	Natural logarithm
LPS	Lipopolysaccharide
LRT	Long range transport
mRNA	Messenger ribonucleic acid
ME	Mercaptoethanol
MEPP	Miniature end-plate potential
MES	Maximal electroshock seizure
MeV	Mega-electron volts
MLC	Mixed lymphocyte culture
MMD	Mass median diameter
MMAD	Mass median aerodynamic diameter
Mn	Manganese
MND	Motor neuron disease
MSV	Moloney sarcoma virus
MTD	Maximum tolerated dose
n	Number of subjects or observations
N/A	Not Available

LIST OF ABBREVIATIONS

NA	Not Applicable
NAAQS	National ambient air quality standards
NAD	Nicotinamide Adenine Dinucleotide
NADB	National Aerometric Data Bank
NAMS	National Air Monitoring Station
NAS	National Academy of Sciences
NASN	National Air Surveillance Network
NBS	National Bureau of Standards
NE	Norepinephrine
NFAN	National Filter Analysis Network
NFR-82	Nutrition Foundation Report of 1982
NHANES II	National Health Assessment and Nutritional Evaluation Survey II
Ni	Nickel
NTA	Nitrilotriacetonitrile
OSHA	Occupational Safety and Health Administration
P	Phosphorus
p	Significance symbol
PAH	Para-aminohippuric acid
Pb	Lead
PBA	Air lead
$Pb(Ac)_2$	Lead acetate
PbB	concentration of lead in blood
PbBrCl	Lead (II) bromochloride
PBG	Porphobilinogen
PFC	Plaque-forming cells
pH	Measure of acidity
PHA	Phytohemagglutinin
PHZ	Polyacrylamide-hydrous-zirconia
PIXE	Proton-induced X-ray emissions
PMN	Polymorphonuclear leukocytes
PND	Post-natal day
PNS	Peripheral nervous system
p.o.	Per os (orally)
ppm	Parts per million
PRA	Plasma renin activity
PRS	Plasma renin substrate
PWM	Pokeweed mitogen
Py-5-N	Pyrimide-5'-nucleotidase
RBC	Red blood cell; erythrocyte
RBF	Renal blood flow
RCR	Respiratory control ratios/rates
redox	Oxidation-reduction potential
RES	Reticuloendothelial system
RLV	Rauscher leukemia virus
RNA	Ribonucleic acid
S-HT	Serotonin
SA-7	Simian adenovirus
s.c.	Subcutaneously (method of injection)
scm	Standard cubic meter
S.D.	Standard deviation
SDS	Sodium dodecyl sulfate
S.E.M.	Standard error of the mean

LIST OF ABBREVIATIONS (continued).

SES	Socioeconomic status
SGOT	Serum glutamic oxaloacetic transaminase
sIg	Surface immunoglobulin
SLAMS	State and local air monitoring stations
SMR	Standardized mortality ratio
Sr	Strontium
SRBC	Sheep red blood cells
SRMs	Standard reference materials
STEL	Short-term exposure limit
SW voltage	Slow-wave voltage
T-cells	Thymus-derived lymphocytes
t-tests	Tests of significance
TBL	Tri-n-butyl lead
TEA	Tetraethyl-ammonium
TEL	Tetraethyllead
TIBC	Total iron binding capacity
TML	Tetramethyllead
TMLC	Tetramethyllead chloride
TSH	Thyroid-stimulating hormone
TSP	Total suspended particulate
U.K.	United Kingdom
UMP	Uridine monophosphate
USPHS	U.S. Public Health Service
VA	Veterans Administration
V_d	Deposition velocity
VER	Visual evoked response
WHO	World Health Organization
XRF	X-Ray fluorescence
X^2	Chi squared
Zn	Zinc
ZPP	Erythrocyte zinc protoporphyrin

MEASUREMENT ABBREVIATIONS

dl	deciliter
ft	feet
g	gram
g/gal	gram/gallon
g/ha·mo	gram/hectare·month
km/hr	kilometer/hour
l/min	liter/minute
mg/km	milligram/kilometer
$\mu g/m^3$	microgram/cubic meter
mm	millimeter
μm	micrometer
μmol	micromole
ng/cm^2	nanograms/square centimeter
nm	nanometer
nM	nanomole
sec	second
t	tons

GLOSSARY VOLUME IV

ADP/O ratio - a measure of the rate of respiration; the ratio of adenosine diphosphate concentration to oxygen levels increases as respiration is impaired

active transport - the translocation of a solute across a membrane by means of an energy-dependent carrier system capable of moving against a concentration gradient

affective function - pertaining to emotion

asthenospermia - loss or reduction of the motility of spermatozoa

azotemia - an excess of urea and other nitrogenous compounds in the blood

basal ganglia - all of the large masses of gray matter at the base of the cerebral hemispheres, including the corpus striatum, subthalamic nucleus, and substantia nigra

basophilic stippling - a histochemical appearance characteristic of immature erythrocytes

cognitive function - pertaining to reasoning, judging, conceiving, etc.

corpuscular volume - red blood cell volume

cristae - shelf-like infoldings of the inner membrane of mitochondria

cytomegaly - markedly enlarged cells

demyelination - destruction of the protective myelin sheath which surrounds most nerves

depolarization - the electrophysiological process underlying neural transmission

desaturation kinetic study - a form of kinetic study in which the rate of release of a species from its binding is studied

desquamation - shedding, peeling, or scaling off

disinhibition - removal of a tonic inhibitory effect

endoneurium - the delicate connective tissue enveloping individual nerve fibers within a nerve

erythrocyte - red blood cell

erythropoiesis - the formation of red blood cells

feedback derepression - the deactivation of a repressor

hepatocyte - a parenchymal liver cell

hyalinization - a histochemical marker characteristic of degeneration

hyperkalemia - a greater than normal concentration of potassium ions in the circulating blood

hyperplasia - increased numbers of cells

hypertrophy - increased size of cells

hypochromic - containing less than the normal amount of pigment

hyporeninemic hypoaldosteronism - pertaining to a systemic deficiency of renin and aldosterone

inclusion bodies - any foreign substance contained or entrapped within a cell

isocortex - cerebral cortex

lysosomes - a cytoplasmic, membrane-bound particle containing hydrolyzing enzymes

macrophage - large scavenger cell that ingests bacteria, foreign bodies, etc.

(Na^+, K^+)-ATPase - an energy-dependent enzyme which transports sodium and potassium across cell membranes

natriuresis - enhanced urinary excretion of sodium

normocytic - refers to normal, healthy-looking erythrocytes

organotypic - disease or cell mixture representative of a specific organ

oxidative phosphorylation - the generation of cellular energy in the presence of oxygen

paresis - partial or incomplete paralysis

pathognomic feature - characteristic or indicative of a disease

polymorphonuclear leukocytes - leukocytes (white blood cells) having nuclei of various forms

respiratory control rates (RCRs) - measure of intermediary metabolism

reticulocytosis - an increase in the number of circulating immature red blood cells

synaptogenesis - the formation of neural connections (synapses)

synaptosomes - morphological unit composed of nerve terminals and the attached synapse

teratogenic - affecting the development of an organism

teratospermia - a condition characterized by the presence of malformed spermatozoa

Chapter 12: Biological Effects of Lead Exposure

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Chapter 13: Risk Assessment

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12. BIOLOGICAL EFFECTS OF LEAD EXPOSURE

12.1 INTRODUCTION

As noted in Chapter 2, air quality criteria documents evaluate scientific knowledge of relationships between pollutant concentrations and their effects on the environment and public health. Chapters 3-7 of this document discuss the following: physical and chemical properties of lead; measurement methods for lead in environmental media; sources of emissions; transport, transformation, and fate; and ambient concentrations and other aspects of the exposure of the U.S. population to lead in the environment. Chapter 8 evaluates the projected impact of lead on ecosystems. Chapters 9-11 discuss the following: measurement techniques for lead in biologic media; aspects related to the uptake, distribution, toxicokinetics, and excretion of lead; and the relationship of various external and internal lead exposure indices to each other. This chapter assesses information regarding biological effects of lead exposure, with emphasis on (1) the qualitative characterization of various lead-induced effects and (2) the delineation of dose-effect relationships for key health effects most likely of concern at ambient exposure levels currently encountered by the general population of the United States.

It is clear from the evidence evaluated in this chapter that there exists a continuum of biological effects associated with lead across a broad range of exposure. At rather low levels of lead exposure, biochemical changes, such as the disruption of certain enzymatic activities involved in heme biosynthesis and erythropoietic pyrimidine metabolism, are detectable. With increasing lead exposure, there are sequentially more pronounced effects on heme synthesis and a broadening of lead's effects to additional biochemical and physiological mechanisms in various tissues, such that progressively more severe disruption of the normal functioning of many different organ systems becomes apparent. In addition to impairment of heme biosynthesis, signs of disruption of normal functioning of the erythropoietic and nervous systems are among the earliest effects observed in response to increasing lead exposure. At increasingly higher exposure levels, more severe disruption of the erythropoietic and nervous systems occurs; other organ systems are also affected so as to result in the manifestation of renal effects, disruption of reproductive functions, impairment of immunological functions, and many other biological effects. At sufficiently high levels of exposure, the damage to the nervous system and other effects can be severe enough to result in death or, in some cases of non-fatal lead poisoning, long-lasting sequelae such as permanent mental retardation.

The etiologies of many of the different types of functional disruption of various mammalian organ systems derive (at least in their earliest stages) from lead's effects on certain subcellular organelles, which result in biochemical derangements (e.g., disruption of heme synthesis processes) common to and affecting many tissues and organ systems. Some major effects of lead on subcellular organelles common to numerous organ systems in mammalian species are discussed below in Section 12.2, with particular emphasis on lead effects on mitochondrial functions. Subsequent sections of this chapter discuss biological effects of lead in terms of various organ or physiological systems affected by the element and its compounds (except for Section 12.7, which assesses genotoxic and carcinogenic effects of lead). Additional cellular and subcellular aspects of the biological effects of lead are discussed within respective sections on particular organ systems.

Sections 12.3 to 12.10 have been sequenced generally according to the degree of known vulnerability of each system to lead. Major emphasis is placed first on detailed discussion of the effects of lead on heme synthesis and associated multisystem impacts on several important physiological processes and organ systems. Effects of lead on the two organ systems classically considered to be most sensitive to lead (i.e., the hematopoietic and the nervous systems) are further emphasized in early sections. Subsequent sections then discuss additional effects of lead on the kidney and on reproduction and development (in view of the impact of lead on the fetus and pregnant women, as well as its gametotoxic effects). Genotoxic effects of lead and evidence for possible carcinogenic effects of lead are then reviewed, followed by discussion of the effects of lead on the immune system and, lastly, other organ systems.

This chapter is subdivided mainly according to organ systems to facilitate presentation of information. It must be noted, however, that in reality, all systems function in delicate concert to preserve the physiological integrity of the whole organism and all systems are interdependent. Thus, not only do effects in a critical organ often exert impacts on other organ systems, but low-level effects that might be construed as unimportant in a single specific system may be of concern in terms of their cumulative or aggregate impact.

Special emphasis is placed on the discussion of the effects of lead exposure in children. Children are particularly at risk due to sources of exposure, mode of entry, rate of absorption and retention, and partitioning of lead in soft and hard tissues. The greater sensitivity of children to lead toxicity, their inability to recognize symptoms, and their dependence on parents and health care professionals make them an especially vulnerable population requiring special consideration in developing criteria and standards for lead.

In discussing the biological effects of lead, it is important to note that lead has not been demonstrated to have any beneficial biological effect in humans. Some recent studies have raised the possibility that lead could be a nutritionally essential element. The primary evidence for this view has come from a series of articles by Kirchgessner and Reichlmayr-Lais, who have reported that rats maintained on a semi-synthetic diet low in lead (either 18 or 45 ppb) over several generations showed reduced growth rates (Reichlmayr-Lais and Kirchgessner, 1981a), disturbances in hematological indices, tissue iron, and iron absorption

(Reichlmayr-Lais and Kirchgessner, 1981b,c,d,e; Kirchgessner and Reichlmayr-Lais, 1981a,b), and changes in certain enzyme activities and metabolite levels (Reichlmayr-Lais and Kirchgessner, 1981f; Kirchgessner and Reichlmayr-Lais, 1982). Diets containing 18 ppb lead were associated with the most pronounced effects on iron metabolism and growth as well as on enzyme activities and metabolite levels. Animals in the F_1-group maintained on a 45-ppb lead diet showed moderate changes in some hematological indices.

These studies were reviewed by a committee of independent scientists convened by the U.S. Environmental Protection Agency (Expert Committee on Trace Metal Essentiality, 1983). The Committee's conclusions were as follows:

1. The Kirchgessner and Reichlmayr-Lais data furnish evidence that is consistent with and, in some opinions, indicative of a nutritional essentiality of lead for rats.
2. The evidence is not sufficient to establish nutritional essentiality of lead for rats.
3. To address the basic issue of nutritional essentiality of lead, additional evidence needs to be obtained under different conditions in the laboratory of Kirchgessner and Reichlmayr-Lais, as well as by independent investigators; additional species should also be examined.

The Committee emphasized the difference that apparently exists between lead concentrations that are of concern from a toxicologic viewpoint (e.g., those associated with effects of the various types discussed in this chapter) and much lower lead levels that might possibly be of nutritional value. Hence the Committee did not perceive any practical incompatibility between (a) efforts to reduce lead in the human environment to concentrations that are unassociated with toxic effects and (b) efforts to define the potential nutritional essentiality of lead. The Committee further recognized that current public health concerns for humans clearly focus on lead toxicity effects.

Finally, the question of lead essentiality is largely moot in the debate over lead as a public health issue. The extent of permanent and pervasive lead contamination in developed areas of the world is such that concern will remain with excessive lead exposure and associated toxicity in human populations. It is virtually inconceivable that lead deficiency in human populations would ever arise in the first place.

12.2 SUBCELLULAR EFFECTS OF LEAD IN HUMANS AND EXPERIMENTAL ANIMALS

The biochemical or molecular basis for lead toxicity is the ability of the toxicant, as a metallic cation, to bind to ligating groups in biomolecular substances crucial to normal physiological functions, thereby interfering with these functions via such mechanisms as competition with native essential metals for binding sites, inhibition of enzyme activity, and inhibition or other alterations of essential ion transport. The relationship of this basis for lead toxicity to organ- and organelle-specific effects is modulated by the following: (1) the inherent stability of such binding sites for lead; (2) the compartmentalization kinetics governing lead distribution among body compartments, among tissues, and within cells; and (3) differences in biochemical and physiological organization across tissues and cells due to their specific function. Given complexities introduced by factors 2 and 3, it is not surprising that no single, unifying mechanism of lead toxicity has been demonstrated to apply across all tissues and organ systems.

In the 1977 Air Quality Criteria Document for Lead, cellular and subcellular effects of lead were discussed, including effects on various classes of enzymes. Much of the literature detailing the effects of lead on enzymes *per se* has entailed study of relatively pure enzymes *in vitro* in the presence of added lead. This was the case for data discussed in the earlier document and such information continues to appear in the literature. Much of this material is of questionable relevance for effects of lead *in vivo*. On the other hand, lead effects on certain enzymes or enzyme systems are recognized as integral mechanisms of action underlying the impact of lead on tissues *in vivo* and are logically discussed in later sections below on effects at the organ system level.

This subsection is mainly concerned with organellar effects of lead, especially those that provide some rationale for lead toxicity at higher levels of biological organization. While a common mechanism at the subcellular level that would account for all aspects of lead toxicity has not been identified, one fairly common cellular response to lead is the impairment of mitochondrial structure and function; thus, mitochondrion effects are accorded major attention here. Lead effects on other organelles have not been as extensively studied as mitochondrion effects; in some cases, it is not clear how the available information, e.g., that on lead-containing nuclear inclusion bodies, relates to organellar dysfunction.

12.2.1 Effects of Lead on the Mitochondrion

The mitochondrion is clearly the target organelle for toxic effects of lead on many tissues, the characteristics of vulnerability varying somewhat with cell type. Given early recognition of this sensitivity, it is not surprising that an extensive body of *in vivo* and *in vitro* data has accumulated, which can be characterized as evidence of the following: (1) structural injury to the mitochondrion; (2) impairment of basic cellular energetics and other mitochondrial functions; and (3) uptake of lead by mitochondria *in vivo* and *in vitro*.

12.2.1.1 Lead Effects on Mitochondrial Structure. Changes in mitochondrial morphology with lead exposure have been well documented in humans and experimental animals and, in the case of the kidney, are a rather early response to such exposure. Earlier studies have been reviewed by Goyer and Rhyne (1973), followed by later updates by Fowler (1978) and Bull (1980).

Chronic oral exposure of adult rats to lead (1 percent lead acetate in diet) results in structural changes in renal tubule mitochondria, including swelling with distortion or loss of cristae (Goyer, 1968). Such changes have also been documented in renal biopsy tissue of lead workers (Wedeen et al., 1975; Biagini et al., 1977) and in tissues other than kidney, i.e., heart (Malpass et al., 1971; Moore et al., 1975b), liver (Hoffmann et al., 1972), and the central (Press, 1977) and peripheral (Brashear et al., 1978) nervous systems.

While it appears that relatively high-level lead exposures are necessary to detect structural changes in mitochondria in some animal models (Goyer, 1968; Hoffmann et al., 1972), changes in rat heart mitochondria have been seen at blood lead levels as low as 42 μg/dl. Also, in the study of Fowler et al. (1980), swollen mitochondria in renal tubule cells were seen in rats chronically exposed to lead from gestation to 9 months of age at a dietary lead dosing level as low as 50 ppm and a median blood lead level of 26 μg/dl (range: 15-41 μg/dl). Taken collectively, these differences point out relative tissue sensitivity, dosing protocol, relative sensitivity of the various experimental techniques, and the possible effect of developmental status (Fowler et al., 1980) as important factors in determining lead exposure levels at which mitochondria are affected in various tissues.

12.2.1.2 Lead Effects on Mitochondrial Function. Both *in vivo* and *in vitro* studies dealing with such effects of lead as the impact on energy metabolism, intermediary metabolism, and intracellular ion transport have been carried out in various experimental animal models. Of particular interest for this section are such effects of lead in the developing versus the adult animal, given the greater sensitivity of the young organism to lead.

12.2.1.3 In Vivo Studies. Uncoupled energy metabolism, inhibited cellular respiration using succinate and nicotinamide adenine dinucleotide (NAD)-linked substrates, and altered kinetics of intracellular calcium have all been documented for animals exposed to lead *in vivo*, as reviewed by Bull (1980).

Adult rat kidney mitochondria, following chronic oral feeding of lead in the diet (1 percent lead acetate, 10 or more weeks) showed a marked sensitivity of the pyruvate-NAD reductase system (Goyer, 1971), as indicated by impairment of pyruvate-dependent respiration indexed by ADP/O ratio and respiratory control rates (RCRs). Succinate-mediated respiration in these animals, however, was not different from controls. In contrast, Fowler et al. (1980) found in rats exposed *in utero* (dams fed 50 or 250 ppm lead) and for 9 months postnatally (50 or 250 ppm lead in their diet) renal tubule mitochondria that exhibited decreased state 3 respiration and RCRs for both succinate and pyruvate/malate substrates. This may have been due to longer exposure to lead or a differential effect of lead exposure during early development.

Intraperitoneal administration of lead to adult rats at doses as low as 12 mg/kg over 14 days was associated with inhibition of potassium-stimulated respiration in cerebral cortex slices with impairment of NADPH (NAD phosphate, reduced) oxidation using glucose, but not pyruvate, as substrate (Bull et al., 1975). This effect occurred at a corresponding blood lead of 72 µg/dl and a brain lead content of 0.4 µg/g, values below those associated with overt neurotoxicity. Bull (1977), in a later study, demonstrated that the respiratory response of cerebral cortical tissue from lead-dosed rats receiving a total of 60 mg/kg (10 mg/kg x 6 dosings) over 14 days was associated with a marked decrease in turnover of intracellular calcium in a cellular compartment that appears to be the mitochondrion. This is consistent with the observation of Bouldin et al. (1975) that lead treatment leads to increased retention of calcium in guinea pig brain.

Numerous studies have evaluated relative effects of lead on mitochonodria of developing versus adult animals, particularly in the nervous system. Holtzman and Shen Hsu (1976) exposed rat pups at 14 days of age to lead via milk of mothers fed lead in the diet (4 percent lead carbonate) with exposure lasting for 14 days. A 40 percent increase in state 4 respiratory rate of cerebellar mitochonodria was seen within one day of treatment and was lost at the end of the exposure period. However, at this later time (28 days of age), a substantial inhibition of state 3 respiration was observed. This early effect of lead on uncoupling oxidative phosphorylation is consistent with the results of Bull et al. (1979) and McCauley et al. (1979). In these investigations, female rats received lead in drinking water (200 ppm) from 14 days before breeding through weaning of the pups. At 15 days of age, cerebral cortical slices showed alteration of potassium-stimulated respiratory response and glucose uptake.

Holtzman et al. (1980a) compared mitochondrial respiration in cerebellum and cerebrum in rat pups exposed to lead beginning at 14 days of age (via milk of mothers fed 4 percent lead carbonate) and in adult rats maintained on the same diet. Cerebellar mitochondria showed a very early loss (by 2 days of exposure) of respiratory control in the pups with inhibition of phosphorylation-coupled respiration for NAD-linked substrates but not for succinate. Such changes were less pronounced in mitochondria of the cerebrum and were not seen for either brain region in the adult rat. This regional-and age-dependency of mitochondrial impairment parallels features of lead encephalopathy.

In a second study addressing this issue, Holtzman et al. (1981) measured the cytochrome contents of cerebral and cerebellar mitochondria from rat pups exposed either from birth or at 14 days of age via the same dosing protocol noted above. These were compared to adult animals exposed in like fashion. Pups exposed to lead from birth showed statistically significant reductions of cytochrome b, cytochromes c + c_1, and cytochromes a + a_3 in cerebellum by 4 weeks of exposure. Changes in cerebral cytochromes, in contrast, were marginal. When lead exposure began at 14 days of age, little effect was observed, and adult rats showed little change. This study indicates that the most vulnerable period for lead effects on developing brain oxidative metabolism is the same period where a major burst in such activity begins.

Related to effects of lead on energy metabolism in the developing animal mitochondrion is the effect on brain development. In the study of Bull et al. (1979) noted earlier, cerebral cytochrome c + c_1 levels between 10 and 15 days of age decreased in a dose-dependent fashion at all maternal dosing levels (5-100 mg Pb/liter drinking water) and corresponding blood lead values for the rat pups (11.7-35.7 μg/dl). Delays in synaptic development in these pups also occurred, as indexed by synaptic counts taken in the parietal cortex. As the authors concluded, uncoupling of energy metabolism appears to be causally related to delays in cerebral cortical development.

Consistent with the effects of lead on mitochondrial structure and function are in vivo data demonstrating the selective accumulation of lead in mitochondria. Studies in rats using radioisotopic tracers ^{210}Pb (Castellino and Aloj, 1969) and ^{203}Pb (Barltrop et al., 1971) demonstrate that mitochondria will accumulate lead in significant relative amounts, the nature of the accumulation seeming to vary with the dosing protocol. Sabbioni and Marafante (1976) as well as Murakami and Hirosawa (1973) also found that lead is selectively lodged in mitochondria. Of interest in regard to the effects of lead on brain mitochondria are the data of Moore et al. (1975a) showing uptake of lead by guinea pig cerebral mitochondria, and the results of Krigman et al. (1974c) demonstrating that mitochondria in brain from 6-month-old rats exposed chronically to lead since birth showed the highest uptake of lead (34 percent), followed by the nuclear fraction (31 percent). While the possibility of translocation of lead during subcellular fractionation can be raised, the distribution pattern seen in the reports of Barltrop et al. (1971) and Castellino and Aloj (1969) over multiple time points makes this unlikely. Also, findings of in vivo uptake of lead in brain mitochondria are supported by in vitro data discussed below.

12.2.1.4 In Vitro Studies. In vitro studies of both the response of mitochondrial function to lead and its accumulation by the organelle have been reported, using both isolated mitochondria and tissues. Bull (1980) reviewed such data published up to 1979.

Significant reductions in mitochondrial respiration, using both NAD-linked and succinate substrates, have been reported for isolated heart and brain mitochondria. The lowest levels of lead associated with such effects appear to be 5 μM or, in some cases, less. Available evidence suggests that the sensitive site for lead in isolated mitochondria is before cytochrome b in the oxidative chain and involves either tricarboxylic acid enzymes or non-heme protein/ubiquinone steps. If substrate specificity is compared, e.g., succinate versus glutamate/malate oxidation, there appear to be organ-specific differences. As Bull (1980) noted, tissue-specific effects of lead on cellular energetics may be one basis for differences in toxicity across organs. Also, several enzymes involved in intermediary metabolism in isolated mitochondria have been observed to undergo significant inhibition of activity in the presence of lead; these have been tabulated by Bull (1980).

One focus of studies dealing with lead effects on isolated mitochondria has been ion transport--particularly that of calcium. Scott et al. (1971) have shown that lead movement into rat heart mitochondria involves active transport, with characteristics similar to those of calcium, thereby establishing a competitive relationship. Similarly, lead uptake into brain mitochondria is also energy-dependent (Holtzman et al., 1977; Goldstein et al., 1977). The recent elegant studies of Pounds and coworkers (Pounds et al., 1982a,b), using labeled calcium or lead and desaturation kinetic studies of these labels in isolated rat hepatocytes, have elucidated the intracellular relationship of lead to calcium in terms of cellular compartmentalization. In the presence of graded amounts of lead (10, 50, or 100 μM), the kinetic analysis of desaturation curves of ^{45}Ca label showed a lead dose-dependent increase in the size of all three calcium compartments within the hepatocyte, particularly that deep compartment associated with the mitochondrion (Pounds et al., 1982a). Such changes were seen to be relatively independent of serum calcium or endogenous regulators of systemic calcium metabolism. Similarly, the use of ^{210}Pb label and analogous kinetic analysis (Pounds et al., 1982b) showed the same three compartments of intracellular distribution as noted for calcium, including the deep component A: redundant. Hence, there is striking overlap in the cellular metabolism of calcium and lead. These studies not only further confirm easy entry of lead into cells and cellular compartments, but also provide a basis for perturbation by lead of intracellular ion transport, particularly in neural cell mitochondria, where there is a high capability for calcium transport. Such capability is approximately 20-fold higher than in heart mitochondria (Nicholls, 1978).

Given the above observations, it is not surprising that a number of investigators have noted the in vitro uptake of lead into isolated mitochondria. Walton (1973) noted that lead is accumulated within isolated rat liver mitochondria over the range of 0.2-100 μM lead; Walton and Buckley (1977) extended this observation to mitochondria in rat kidney cells in culture. Electron microprobe analyses of isolated rat synaptosomes (Silbergeld et al., 1977) and capillaries (Silbergeld et al., 1980b) incubated with lead ion have established that significant accumulation of lead, along with calcium, occurs in the mitochondrion. These observations are consistent with the kinetic studies of Pounds et al. (1982a,b), and the in vitro data for isolated capillaries are in accord with the observations of Toews et al. (1978), who found significant lead accumulation in brain capillaries of the suckling rat.

12.2.2 Effects of Lead on the Nucleus

With lead exposure, a cellular reaction typical of many species (including humans) is the formation of intranuclear lead-containing inclusion bodies, early data for which have been summarized by Goyer and Moore (1974). In brief, these lead-bearing inclusion bodies A: (1) have have been verified as to lead content by X-ray microanalysis (Carroll et al., 1970);

(2) consist of a rather dense core encapsulated by a fibrillary envelope; (3) are a complex of lead and the acid fractions of nuclear protein; (4) can be disaggregated *in vitro* by EDTA; (5) can appear very rapidly after lead exposure (Choie et al., 1975); (6) consist of a protein moiety in the complex which is synthesized *de novo*; and (7) have been postulated to serve a protective role in the cell, given the relative amounts of lead accumulated and presumably rendered toxicologically inert.

Based on renal biopsy studies, Cramer et al. (1974) concluded that such inclusion body formation in renal tubule cells in lead workers is an early response to lead entering the kidney, in view of decreased presence of the inclusion bodies as a function of increased period of employment. Schumann et al. (1980), however, observed a continued exfoliation of inclusion-bearing tubule cells into urine of workers having a variable employment history.

Any protective role played by the lead inclusion body appears to be an imperfect one, to the extent that both subcellular organelle injury and lead uptake occur simultaneously with such inclusion formation, often in association with severe toxicity at the organ system level. For example, Osheroff et al. (1982) observed lead inclusion bodies in the anterior horn cells of the cervical spinal cord and neurons of the substantia nigra (as well as in renal tubule cells) in the adult rhesus monkey, along with damage to the vascular walls and glial processes and ependymal cell degeneration. At the light- and electron-microscope level, there were no signs of neuronal damage or altered morphology except for the inclusions. As noted by the authors, these inclusions in the large neurons of the substantia nigra show that the neuron will take up and accumulate lead. In the study of Fowler et al. (1980), renal tubule inclusions were observed simultaneously with evidence of structural and functional damage to the mitochondrion, all at relatively low levels of lead. Hence, it appears that a limited protective role for these inclusions may extend across a range of lead exposure.

Chromosomal effects and other indices of genotoxicity in humans and animals are discussed in Section 12.7 of this chapter.

12.2.3 Effects of Lead on Membranes

In theory, the cell membrane is the first organelle to encounter lead, and it is not surprising that cellular effects can be ascribed to interactions at cellular and intracellular membranes, mainly appearing to be associated with ion transport processes across membranes. In Section 12.3 a more detailed discussion is accorded the effects of lead on the membrane as they relate to the erythrocyte in terms of increased cell fragility and increased osmotic resistance. These effects can be rationalized, in large part, by the documented inhibition by lead of erythrocyte membrane (Na^+, K^+)-ATPase.

Lead also appears to interfere with the normal processes of calcium transport across membranes of various tissue types. Silbergeld and Adler (1978) have described lead-induced retardation of the release of the neurotransmitter, acetylcholine, in peripheral cholinergic synaptosomes, due to a blockade of calcium binding to the synaptosomal membrane, reducing calcium-dependent choline uptake and subsequent release of acetylcholine from the nerve terminal. Calcium efflux from neurons is mediated by the membrane (Na^+, K^+)-ATPase via an exchange process with sodium. Inhibition of the enzyme by lead, as also occurs with the erythroctye (see above), increases the concentration of calcium within nerve endings (Goddard and Robinson, 1976). As seen from the data of Pounds et al. (1982a), lead can also elicit retention of calcium in neural cells by easy entry into the cell and by directly affecting the deep calcium compartment within the cell, of which the mitochondrion is a major component.

12.2.4 Other Organellar Effects of Lead

Studies of morphological alterations of renal tubule cells in the rat (Chang et al., 1981) and rabbit (Spit et al., 1981) with varying lead treatments have demonstrated lead-induced lysosomal changes. In the rabbit, with relatively modest levels of lead exposure (0.25 or 0.5 mg/kg, 3 times weekly over 14 weeks) and corresponding blood lead values of 50 and 60 µg/dl, there was a dose-dependent increase in the amount of lysosomes in proximal convoluted tubule cells, as well as increased numbers of lysosomal inclusions. In the rat, exposure to 10 mg/kg i.v. (daily over 4 weeks) resulted in the accumulation of lysosomes, some gigantic, in the pars recta segment of renal tubules. These animal data are consistent with observations made in lead workers (Cramer et al., 1974; Wedeen et al., 1975) and appear to represent a disturbance in normal lysosomal function, with the accumulation of lysosomes being due to enhanced degradation of proteins arising from effects of lead elsewhere within the cell.

12.2.5 Summary of Subcellular Effects of Lead

The biological basis of lead toxicity is closely linked to the ability of lead to bind to ligating groups in biomolecular substances crucial to normal physiological functions. This binding interferes with physiological processes by such mechanisms as the following: competition with native essential metals for binding sites, inhibition of enzyme activity, and inhibition or other changes in essential ion transport.

The main target organelle for lead toxicity in a variety of cell and tissue types clearly is the mitochondrion, followed probably by cellular and intracellular membranes. Mitochondrial effects take the form of structural changes and marked disturbances in mitochondrial function within the cell, especially energy metabolism and ion transport. These effects are associated, in turn, with demonstrable accumulation of lead in mitochondria, both *in vivo* and *in vitro*. Structural changes include mitochondrial swelling in many cell types, as well as distortion and loss of cristae, which occur at relatively moderate levels of lead exposure. Similar changes have been documented in lead workers across a wide range of exposure levels.

Uncoupled energy metabolism, inhibited cellular respiration using both succinate and nicotinamide adenine dinucleotide (NAD)-linked substrates, and altered kinetics of intracellular calcium have been demonstrated _in vivo_ using mitochondria of brain and non-neural tissue. In some cases, relatively moderate lead exposure levels have been associated with such changes, and several studies have documented the relatively greater sensitivity of this organelle in young versus adult animals in terms of mitochondrial respiration. The cerebellum appears to be particularly sensitive, providing a connection between mitochondrial impairment and lead encephalopathy. Impairment by lead of mitochondrial function in the developing brain has also been associated with delayed brain development, as indexed by content of various cytochromes. In the rat pup, ongoing lead exposure from birth is required for this effect to be expressed, indicating that such exposure must occur before, and is inhibitory to, the burst of oxidative metabolism activity that normally occurs in the young rat 10-21 days postnatally.

In vivo lead exposure of adult rats has also been observed to markedly inhibit cerebral cortex intracellular calcium turnover (in a cellular compartment that appears to be the mitochondrion) at a brain lead level of 0.4 ppm. These results are consistent with a separate study showing increased retention of calcium in the brains of lead-dosed guinea pigs. A number of reports have described the _in vivo_ accumulation of lead in mitochondria of kidney, liver, spleen, and brain tissue, with one study showing that such uptake was slightly more than occurred in the nucleus. These data are not only consistent with the various deleterious effects of lead on mitochondria but are also supported by other, _in vitro_ findings.

Significant decreases in mitochondrial respiration _in vitro_, using both NAD-linked and succinate substrates, have been observed for brain and non-neural tissue mitochondria in the presence of lead at micromolar levels. There appears to be substrate specificity in the inhibition of respiration across different tissues, which may be a factor in differential organ toxicity. Also, a number of enzymes involved in intermediary metabolism in isolated mitochondria have been observed to undergo significant inhibition of activity with lead.

A major focus of research on lead effects on isolated mitochondria has concerned ion (especially calcium) transport. Lead movement into brain and other tissue mitochondria, as does calcium movement, involves active transport. Recent sophisticated kinetic analyses of desaturation curves for radiolabeled lead or calcium indicate that there is striking overlap in the cellular metabolism of calcium and lead. These studies not only establish a basis for easy entry of lead into cells and cell compartments, but also provide a basis for impairment by lead of intracellular ion transport, particularly in neural cell mitochondria, where the capacity for calcium transport is 20-fold higher than even in heart mitochondria.

Lead is also selectively taken up in isolated mitochondria *in vitro*, including the mitochondria of synaptosomes and brain capillaries. Given the diverse and extensive evidence of lead's impairment of mitochondrial structure and function as viewed from a subcellular level, it is not surprising that these derangements are logically held to be the basis of dysfunction of heme biosynthesis, erythropoiesis, and the central nervous system. Several key enzymes in the heme biosynthetic pathway are intramitochondrial, particularly ferrochelatase. Hence, it is to be expected that entry of lead into mitochondria will impair overall heme biosynthesis, and in fact this appears to be the case in the developing cerebellum. Furthermore, the levels of lead exposure associated with entry of lead into mitochondria and expression of mitochondrial injury can be relatively moderate.

Lead exposure provokes a typical cellular reaction in human and other species that has been morphologically characterized as a lead-containing nuclear inclusion body. Although it has been postulated that such inclusions constitute a cellular protection mechanism, such a mechanism is an imperfect one. Other organelles, e.g., the mitochondrion, also take up lead and sustain injury in the presence of nuclear inclusion bodies. Chromosomal effects and other indices of genotoxicity in humans and animals are considered later, in Section 12.7.

In theory, the cell membrane is the first organelle to encounter lead and it is not surprising that cellular effects of lead can be ascribed to interactions at cellular and intracellular membranes in the form of disturbed ion transport. The inhibition of membrane (Na^+,K^+)-ATPase of erythrocytes as a factor in lead-impaired erythropoiesis is noted elsewhere. Lead also appears to interfere with the normal processes of calcium transport across membranes of different tissues. In peripheral cholinergic synaptosomes, lead is associated with retarded release of acetylcholine owing to a blockade of calcium binding to the membrane, while calcium accumulation within nerve endings can be ascribed to inhibition of membrane (Na^+,K^+)-ATPase.

Lysosomes accumulate in renal proximal convoluted tubule cells of rats and rabbits given lead over a wide range of dosing. This also appears to occur in the kidneys of lead workers and seems to represent a disturbance in normal lysosomal function, with the accumulation of lysosomes being due to enhanced degradation of proteins because of the effects of lead elsewhere within the cell.

Insofar as effects of lead on the activity of various enzymes are concerned, many of the available studies concern *in vitro* behavior of relatively pure enzymes with marginal relevance to various effects *in vivo*. On the other hand, certain enzymes are basic to the effects of lead at the organ or organ system level, and discussion is best reserved for such effects in ensuing sections of the document dealing with these systems.

12.3 EFFECTS OF LEAD ON HEME BIOSYNTHESIS AND ERYTHROPOIESIS/ERYTHROCYTE PHYSIOLOGY IN HUMANS AND ANIMALS

Lead has well-recognized effects not only on heme biosynthesis, a crucial process common to many organ systems, but also on the formation and physiology of erythrocytes. This section is therefore divided for purposes of discussion into the following: (1) effects of lead on heme biosynthesis including discussion of interrelationships between heme biosynthesis impairment and (a) interference with vitamin-D metabolism and (b) certain neurotoxic effects of lead; and (2) effects of lead on erythropoiesis and erythrocyte physiology. Discussion of the latter is further subdivided into effects of lead on hemoglobin production, cell morphology and survival, and erythropoietic nucleotide metabolism.

12.3.1 Effects of Lead on Heme Biosynthesis

The effects of lead on heme biosynthesis are very well known because of their prominence and the large number of studies of these effects in humans and experimental animals. In addition to being a constituent of hemoglobin, heme is a prosthetic group of a number of tissue hemoproteins having diverse functions, such as myoglobin, the P-450 component of the mixed-function oxidase system, and the cytochromes of cellular energetics. Hence, any effects of lead on heme biosynthesis will, perforce, pose the potential for multi-organ toxicity.

At present, much of the available information concerning the effects of lead on heme biosynthesis has been obtained by measurements in blood, due in large part to the relative ease of access to blood and in part to the fact that blood is the vehicle for movement of metabolites from other organ systems. On the other hand, a number of reports have been concerned with lead effects on heme biosynthesis in tissues such as kidney, liver, and brain. In the discussion below, various steps in the heme biosynthetic pathway affected by lead are discussed separately, with information describing erythropoietic effects usually appearing first, followed by studies involving other tissues.

The process of heme biosynthesis results in formation of a porphyrin, protoporphyrin IX, starting with glycine and succinyl-coenzyme A. Heme biosynthesis culminates with the insertion of iron at the center of the porphyrin ring. As may be noted in Figure 12-1, lead interferes with heme biosynthesis by disturbing the activity of three major enzymes: (1) it indirectly stimulates, by feedback derepression, the mitochondrial enzyme delta-aminolevulinic acid synthetase (ALA-S), which mediates the condensation of glycine and succinyl-coenzyme A to form delta-aminolevulinic acid (ALA); (2) it directly inhibits the cytosolic enzyme delta-aminolevulinic acid dehydrase (ALA-D), which catalyzes the cyclocondensation of two units of ALA to porphobilinogen; (3) it disturbs the mitochondrial enzyme ferrochelatase, found in

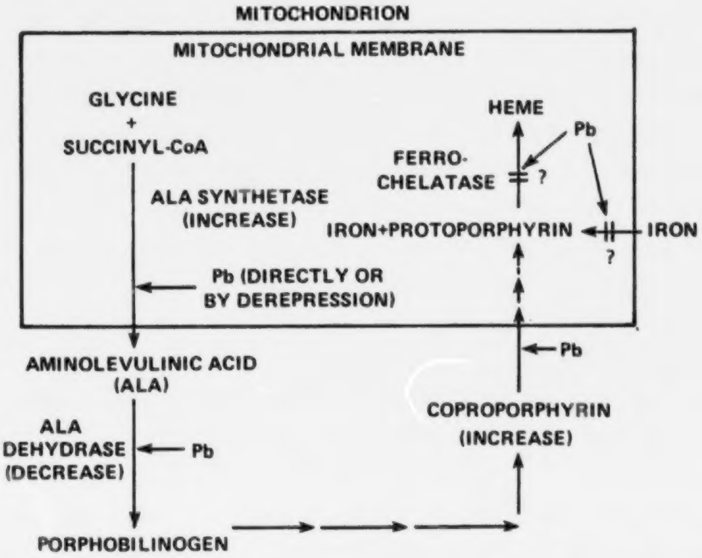
MITOCHONDRION
MITOCHONDRIAL MEMBRANE
GLYCINE
+
SUCCINYL-CoA
ALA SYNTHETASE
(INCREASE)
Pb (DIRECTLY OR
BY DEREPRESSION)
HEME
Pb
FERRO-
CHELATASE
?
IRON+PROTOPORPHYRIN
IRON
?
Pb
AMINOLEVULINIC ACID
(ALA)
ALA
DEHYDRASE
(DECREASE)
Pb
COPROPORPHYRIN
(INCREASE)
PORPHOBILINOGEN

Figure 12-1. Effects of lead (Pb) on heme biosynthesis.

liver, bone marrow, and other tissues, either by direct inhibition or by alteration of intramitochondrial transport of iron. Ferrochelatase catalyzes the insertion of iron (II) into the protoporphyrin ring to form heme and is situated in mammals in the inner mitochondrial membrane (McKay et al., 1969).

12.3.1.1 Effects of Lead on Delta-Aminolevulinic Acid Synthetase. The activity of the enzyme ALA-S is the rate-limiting step in the heme biosynthetic pathway. With decreased heme formation at other steps downstream or with increased heme oxygenase activity, a compensatory increase of ALA-S activity occurs through feedback derepression and enhances the rate of heme formation. Hence, excess ALA formation is due to both stimulation of ALA-S and direct inhibition of ALA-D (see below).

Increased ALA-S activity has been reported in lead workers (Takaku et al., 1973; Campbell et al., 1977; Meredith et al., 1978), with leukocyte ALA-S reported to be stimulated at a blood lead value of 40 μg/dl (Meredith et al., 1978), a level at which ALA-D activity is significantly inhibited. To the extent that mitochondria in leukocytes show a dose-effect relationship comparable to the bone marrow and hepatic systems, it appears that most of the excess

ALA formation below the observed threshold value is due to ALA-D inhibition. From the authors' data, blood ALA had increased about twofold in a subset of the worker population over the blood lead range of 18-40 μg/dl.

In vitro and in vivo experimental data have provided organ-specific results in terms of the direction of the effect of lead on ALA-S activity. Silbergeld et al. (1982) observed that ALA-S activity was increased in kidney with acute lead exposure in rats, while chronic treatment was associated with increased activity of the enzyme in spleen. In liver, however, ALA-S activity was reduced under both acute and chronic dosing. Fowler et al. (1980) reported that renal ALA-S activity was significantly reduced in rats continuously exposed to lead in utero, through development, and up to 9 months of age. Meredith and Moore (1979) noted a steady increase in hepatic ALA-S activity when rats were given lead parenterally over an extended period of time. Maxwell and Meyer (1976) and Goldberg et al. (1978) also noted increased ALA-S activity in rats given lead parenterally. It appears that the type and timeframe of dosing influence the observed effect of lead on the enzyme activity. Using a rat liver cell line (RLC-GAI) in culture, Kusell et al. (1978) demonstrated that lead could produce a time-dependent increase in ALA-S activity. Stimulation of activity was observed at lead levels as low as 5×10^{-6} M, with maximal stimulation at 1×10^{-5} M. The authors reported that the increase in activity was associated with the biosynthesis of more enzyme rather than with stimulation of the pre-existing enzyme. Lead-stimulated ALA-S formation was also not limited to liver cells; rat gliomas and mouse neuroblastomas showed similar results.

12.3.1.2 Effects of Lead on Delta-Aminolevulinic Acid Dehydrase and Delta-Aminolevulinic Acid Accumulation/Excretion. Delta-aminolevulinic acid dehydrase (5-aminolevulinate hydrolase; porphobilinogen synthetase; E.C. 4.2.1.24; ALA-D) is a sulfhydryl, zinc-requiring allosteric enzyme in the heme biosynthetic pathway that catalyzes the conversion of two units of ALA to porphobilinogen. The enzyme's activity is very sensitive to inhibition by lead, but the inhibition is reversed by reactivation of the sulfhydryl group with agents such as dithiothreitol (Granick et al., 1973), zinc (Finelli et al., 1975), or zinc plus glutathione (Mitchell et al., 1977).

The activity of ALA-D appears to be inhibited at virtually all blood lead levels studied so far, and any threshold for this effect remains to be identified (see discussion below). Dresner et al. (1982) found that ALA-D activity in rat bone marrow suspensions was significantly inhibited to 35 percent of control levels in the presence of 5×10^{-7} M (0.5 μM) lead. This potency was unmatched on a comparative molar basis by any other metal tested. Recently, Fujita et al. (1981) showed evidence of an increase in the amount of ALA-D in erythrocytes in lead-exposed rats that was ascribed to an increased rate of ALA-D synthesis in bone marrow cells. Hence, the commonly observed net inhibition of activity occurs even in the face of an increase in ALA-D synthesis.

Hernberg and Nikkanen (1970) found that enzyme activity was correlated inversely with blood lead values in a group of urban, nonexposed subjects. Enzyme activity was inhibited 50 percent at a blood lead level of 16 µg/dl. Other reports have confirmed these observations across age groups and exposure categories (Alessio et al., 1976b; Roels et al., 1975b; Nieburg et al., 1974; Wada et al., 1973). A ratio of activated to inhibited enzyme activity (versus a single activity measurement, which does not accommodate intersubject genetic variability) measured against children's blood lead values of 20-90 µg/dl was employed by Granick et al. (1973) to obtain an estimated threshold of 15 µg/dl for an effect of lead. On the other hand, Hernberg and Nikkanen (1970) observed no threshold in their subjects, all of whom were at or below 16 µg/dl. Note that the lowest blood lead actually measured by Granick et al. (1973) was higher than the values measured by Hernberg and Nikkanen (1970).

Kuhnert et al. (1977) reported that ALA-D activity measures in erythrocytes from both pregnant women and cord blood of infants at delivery are inversely correlated with the corresponding blood lead values, using the activated/inhibited activity ratio method of Granick et al. (1973). The correlation coefficient of activity with lead level was higher in fetal erythrocytes ($r = -0.58$, $p < 0.01$) than in the mothers ($r = -0.43$, $p < 0.01$). The mean inhibition level was 28 percent in mothers versus 12 percent in the newborn. A study by Lauwerys et al. (1978) in 100 pairs of pregnant women and infant cord blood samples confirms this observation, i.e., for fetal blood $r = 0.67$ ($p < 0.001$) and for maternal blood $r = -0.56$ ($p < 0.001$).

While several factors other than lead may affect the activity of erythrocyte ALA-D, much of the available information suggests that most of these factors do not materially compromise the interpretation of the relationship between enzyme activity and lead or the use of this relationship for screening purposes. Border et al. (1976) questioned the reliability of ALA-D activity measurement in subjects concurrently exposed to lead and zinc because zinc also affects the activity of the enzyme. The data of Meredith and Moore (1980) refute this objection. In unexposed subjects who had serum zinc values of 80-120 µM, there was only a minor activating effect with increasing zinc when contrasted to the correlation of activity and blood lead in these same subjects. In workers exposed to both lead and zinc, serum zinc values were greater than in subjects with just lead exposure, but the mean level of enzyme activity was still much lower than in controls ($p < 0.001$).

The preceding discussion indicates that neither variability within the normal range of physiological zinc in humans nor combined excessive zinc and lead exposure in workers materially affects ALA-D activity. The obverse of this, lead exposure in the presence of zinc deficiency, is probably a more significant issue, but one that has not been well studied. Since ALA-D is a zinc-requiring enzyme, one would expect that optimal activity would be governed by *in vivo* zinc availability. Thus, zinc deficiency could potentially have a dual deleterious effect on ALA-D activity: first, a direct reduction in ALA-D activity through reduced zinc availability, and second, an indirect and further inhibition of ALA-D activity because of enhanced lead absorption in the presence of zinc deficiency (see Chapter 10, Section 10.5).

The recent study of Roth and Kirchgessner (1981) indicates that ALA-D activity is significantly decreased in the presence of zinc deficiency. In zinc-deficient rats showing reduced serum and urinary zinc levels, the level of erythrocyte ALA-D activity was only 50 percent that of pair-fed controls, while urinary ALA was significantly elevated. Although these investigators did not measure blood lead in deficient and control animal groups, it would appear that the level of inhibition is more than can be accounted for just on the basis of increased lead absorption from the diet. Given the available information documenting zinc deficiency in children (Section 10.5) as well as the animal study of Roth and Kirchgessner (1981), the relationship of lead, zinc deficiency, and ALA-D activity in young children merits further, careful study.

Moore and Meredith (1979) noted the effects of carbon monoxide on the activity of ALA-D, comparing moderate or heavy smokers with nonsmokers. At the highest level of carboxyhemoglobin measured in their smoker groups, the depression of ALA-D activity was 2.1 percent. In these subjects, a significant inverse correlation of ALA-D activity and blood lead existed, but there was no significant correlation of such activity and blood carboxyhemoglobin levels.

While blood ethanol has been reported to affect ALA-D activity (Moore et al., 1971; Abdulla et al., 1976), its effect is significant only under conditions of acute alcohol intoxication. Hence, relevance of this observation to screening is limited, particularly in children. The effect is reversible, declining with clearing of alcohol from the blood stream.

Lead-induced inhibition of ALA-D activity in erythrocytes apparently reflects a similar effect in other tissues. Secchi et al. (1974) observed a clear correlation in 26 lead workers between hepatic and erythrocyte ALA-D activity as well as the expected inverse correlation between such activity and blood lead in the range of 12-56 µg/dl. In suckling rats, Millar et al. (1970) noted decreased enzyme activity in brain and liver as well as erythrocytes when lead was administered orally. In the study of Roels et al. (1977), tissue ALA-D changes were not observed when dams were administered 1, 10, or 100 ppm lead in drinking water. However, the data of Roels et al. (1977) may reflect a lower effective dose taken in by the dams and delivered to the rat pups in maternal milk, because the pups showed no tissue enzyme activity changes. Silbergeld et al. (1982) described moderate inhibition of ALA-D activity in brain and significant inhibition in kidney, liver, and spleen when adult rats were acutely exposed to lead given intraperitoneally; chronic exposure was associated with reduced activity in kidney, liver, and spleen. Gerber et al. (1978) found that neonatal mice exposed to lead from birth through 17 days of age at a level of 1.0 mg/ml in water showed significant decreases in brain ALA-D activity ($p < 0.01$) at all time points studied. These results support the data of Millar et al. (1970) for the suckling rat. In the study by Millar et al., rats exposed from birth through adulthood only showed significant decreases of brain ALA-D activity at 15 and 30 days; this finding also supports other data for the developing rodent. It would appear, therefore, that brain ALA-D activity is more sensitive to lead in the developing animal than in the adult.

The study of Dieter and Finley (1979) sheds light on the relative sensitivity of ALA-D activity in several regions of the brain and permits comparison of blood versus brain ALA-D activity as a function of lead level. Mallard ducks given a single pellet of lead showed 1 ppm lead in blood, 2.5 ppm lead in liver, and 0.5 ppm lead in brain by 4 weeks. Cerebellar ALA-D activity was reduced by 50 percent at a lead level below 0.5 ppm; erythrocyte enzyme activity was lowered by 75 percent. Hepatic ALA-D activity was comparable to cerebellar activity or somewhat less, although the lead level in the liver was fivefold higher. Cerebellar ALA-D activity was significantly below that for cerebrum. In an avian species, then, at blood lead levels at which erythrocyte ALA-D activity was significantly depressed, activity of the enzyme in cerebellum was even more affected relative to lead concentration.

The inhibition of ALA-D is reflected by increased levels of its substrate, ALA, in urine (Haeger, 1957) as well as in whole blood or plasma (O'Flaherty et al., 1980; Meredith et al., 1978; MacGee et al., 1977; Chisolm, 1968; Haeger-Aronsen, 1960). Cramer et al. (1974) demonstrated that ALA clearance into urine parallels glomerular filtration rate across a range of lead exposures, suggesting that increased urinary output with increasing circulating ALA is associated with decreased tubular reabsorption (Moore et al., 1980). Based on their measurements of plasma and urinary ALA across a range of blood lead in adults, O'Flaherty et al. (1980) calculated a mean fractional reabsorption of 40 percent for ALA. Tubular secretion also occurs. Reabsorption appears to be saturable. In rats, fractional reabsorption was much higher, 90-99 percent.

The detailed study of Meredith et al. (1978), which involved both control subjects and lead workers, indicated that in elevated lead exposure the increase in urinary ALA is preceded by a significant rise in circulating levels of ALA. The overall relationship of plasma ALA to blood lead was exponential and showed a perceptible continuity of the correlation even down to the lowest blood lead value of the control group, 18 μg/dl. The relationship of plasma ALA to urinary levels of the precursor was found to be exponential, indicating that as plasma ALA increases, a greater proportion of ALA undergoes excretion into urine. Inspection of the plot of urinary versus plasma ALA in these subjects shows that the correlation persists down to the plasma ALA concentration corresponding to the lowest blood lead level, 18 μg/dl. These results are contradicted by those of O'Flaherty et al. (1980), who showed no correlation of blood lead with plasma ALA below a value of 40 μg/dl. A key factor in these contradictory studies is the method of ALA measurement. Meredith et al. derived their data from a colorimetric technique that measures ALA as well as other aminoketones, while such aminoketones are not detected in the gas-liquid chromatography method used by O'Flaherty et al. Although the measurements of O'Flaherty et al. are generally more specific for ALA in plasma than any colorimetric technique, their validity at low plasma ALA levels remains to be established in the field (see Chapter 9). The blood (plasma) ALA values reported by Meredith et al. were generally higher than those measured by O'Flaherty et al. and appeared to be high in terms of ALA renal clearance rates. ALA is, however the only aminoketone studied so far that correlates with lead directly. Aminoacetone, also measured in the Meredith et al. study, is a metabolite of an amino acid and is not known to be affected by lead exposure. Thus, notwithstanding a positive bias in absolute ALA values, the relative changes in ALA would appear to provide the most plausible basis for the observed correlation with blood lead levels as low as 18 μg/dl in the study by Meredith et al.

Urinary ALA has been employed extensively as an indicator of excessive lead exposure, particularly in occupational settings (e.g., Davis et al., 1968; Selander and Cramér, 1970; Alessio et al., 1976a). The reliability of this test in initial screening of children for lead exposure has been questioned by Specter et al. (1971) and Blanksma et al. (1969), who pointed out the failure of urinary ALA analysis to detect lead exposure when compared with blood lead values. This is due to the fact that an individual subject will show a wide variation in urinary ALA with random sampling. Chisolm et al. (1976) showed that reliable levels could only be obtained with 24-hr collections. In children with blood lead levels above 40 μg/dl, the relationship of ALA in urine to blood lead becomes similar to that observed in lead workers (see below).

A correlation exists between blood lead and the logarithm of urinary ALA in workers (Meredith et al., 1978; Alessio et al., 1976a; Roels et al., 1975a; Wada et al., 1973; Selander and Cramér, 1970) and in children (National Academy of Sciences, 1972). Selander and Cramér (1970) reported that two different correlation curves were obtained, one for individuals below 40 μg/dl blood lead and a different one for values above this, although the degree of correlation was less than with the entire group. A similar observation has been reported by Lauwerys et al. (1974) from a study of 167 workers with blood lead levels of 10-75 μg/dl.

Meredith et al. (1978) found that the correlation curve for blood ALA versus urinary ALA was linear below a blood lead of 40 μg/dl, as was the relationship of blood ALA to blood lead. Hence, there was also a linear relationship between blood lead and urinary ALA below 40 μg/dl, i.e., a continuation of the correlation below the commonly accepted threshold blood lead value of 40 μg/dl (see below). Tsuchiya et al. (1978) have questioned the relevance of using single correlation curves to describe the blood lead-urinary ALA relationship across a broad range of exposure, because they found that this relationship in workers showing moderate, intermediate, and high lead exposure could be described by three correlation curves with different slopes. This finding is consistent with the observations of Selander and Cramér (1970) as well as the results of Meredith et al. (1978) and Lauwerys et al. (1974). Chisolm et al. (1976) described an exponential correlation between blood lead and urinary ALA in children 5 years old or younger, with blood lead levels ranging from 25 to 75 µg/dl. The upward slope in the regression line appears to be most pronounced at a blood lead level of about 40 µg/dl, but the correlation may persist below this level. In adolescents with blood lead below 40 µg/dl, no clear correlation was observed.

It is apparent from the above reports (Tsuchiya et al., 1978; Meredith et al., 1978; Selander and Cramér, 1970) that circulating ALA and urinary ALA levels are elevated and correlated at blood lead values below 40 µg/dl in humans. These findings are consistent, as shown in the Meredith et al. (1978) study, with the significant and steady increase in ALA-D inhibition concomitant with rising blood levels of ALA, even at blood lead values considerably below 40 µg/dl. Increases of ALA in tissues of experimental animals exposed to lead have also been documented. In the study of Silbergeld et al. (1982), acute administration of lead at a rather high dose to adult rats was associated with an elevation in spleen and kidney ALA compared to that of controls, while in chronic exposure there was a moderate increase in ALA in the brain and a large increase (9-fold to 15-fold) in kidney and spleen. Liver levels with either form of exposure were not materially affected, although there was inhibition of liver ALA-D, particularly in the acute dose group.

12.3.1.3 Effects of Lead on Heme Formation from Protoporphyrin. The accumulation of protoporphyrin in the erythrocytes of individuals with lead intoxication has been recognized since the 1930s (Van den Bergh and Grotepass, 1933), but it has only recently been possible to study this effect through the development of sensitive and specific analytical techniques that permit quantitative measurement. In particular, the development of laboratory microtechniques and the hematofluorometer has allowed the determination of dose-effect relationships as well as the use of such measurements to screen for lead exposure.

In humans under normal circumstances, about 95 percent of the protoporphyrin in circulating erythrocytes is zinc protoporphyrin (ZPP), with the remaining 5 percent present as "free" protoporphyrin (Chisolm and Brown, 1979). Accumulation of protoporphyrin IX in the erythrocytes is the result of impaired iron (II) placement in the porphyrin moiety to form heme, an intramitochondrial process. In lead exposure, the porphyrin acquires a zinc ion in lieu of the native iron; the resulting ZPP is tightly bound in the available heme pockets for the life of the erythrocyte, about 120 days (Lamola et al., 1975a,b). Hammond and coworkers (1985) recently observed that in a group of young children aged 3-36 months (n = 165) the fraction of ZPP versus total erythrocyte protoporphyrin (EP) varied with age; it was at a minimum at 3 months and approached unity at 33 months. The basis for the age-related instability of this ratio may be either a biological factor or an artifact of analytical methodology. A plausible biological basis is that zinc bioavailability and zinc nutritional status (suboptimal in the early age groups) determine the extent of zinc placement in EP. The significance of these observations for EP screening of very young children has been noted in Chapter 9.

In lead poisoning, the accumulation of protoporphyrin differs from that seen in the genetically transmitted disorder erythropoietic protoporphyria. In the latter case, there is a defect in ferrochelatase function, i.e., enzyme function is only 10-25 percent of normal (Bloomer, 1980), leading to loose attachment of the porphyrin, accumulated without uptake of zinc, on the surface of the hemoglobin. Loose attachment permits diffusion into plasma and ultimately into the skin, where photosensitivity is induced. This behavior is absent in lead-associated porphyrin accumulation. The two forms of porphyrin, free and zinc-containing, differ sufficiently in fluorescence spectra to permit a laboratory distinction. With iron deficiency, there is also accumulation of protoporphyrin as the zinc complex in the heme pocket; this resembles in large measure the characteristics of lead intoxication.

The elevation of erythrocyte ZPP has been extensively documented as exponentially correlated with blood lead in children (Piomelli et al., 1973; Kammholz et al., 1972; Sassa et al., 1973; Lamola et al., 1975a,b; Roels et al., 1976) and in adult workers (Valentine et al., 1982; Lilis et al., 1978; Grandjean and Lintrup, 1978; Alessio et al., 1976b; Roels et al., 1975a, 1979; Lamola et al., 1975a,b). Reigart and Graber (1976) and Levi et al. (1976) have demonstrated that ZPP elevation can predict which children tend to increase their blood lead levels, a circumstance that probably rests on the nature of chronic lead exposure in certain groups of young children where a pulsatile blood lead curve is superimposed on some level of ongoing intake of lead that continues to elevate the ZPP values.

Accumulation of ZPP only occurs in erythrocytes formed during lead's presence in erythropoietic tissue. This results in a lag of several weeks before the fraction of new ZPP-rich cells is large enough to influence total cell ZPP level. On the other hand, elevated ZPP in erythrocytes long after significant lead exposure has ceased appears to be a better indicator of resorption of stored lead in bone than other measurements. Alessio et al. (1976b) reported that former lead workers removed from exposure at the workplace for more than 12 months in all cases still showed the typical logarithmic correlation between ZPP and blood or urinary lead. However, the best correlation was observed between ZPP and chelatable lead, that fraction of the total body burden considered toxicologically active (see Chapter 10). This post-exposure relationship for adults clearly indicates that significant levels of hematologically toxic lead continue to circulate long after exposure to lead has ceased.

In a report relevant to the problem of multiple-indicator measurements in the assessment of the degree of lead exposure, Hesley and Wimbish (1981) studied changes in blood lead and ZPP in two groups: newly exposed lead workers and those removed from significant exposure. In new workers, blood lead achieved a plateau at 9-10 weeks, while ZPP continued to rise over the entire study interval of 24 weeks. Among workers removed from exposure, both blood lead and ZPP values remained elevated up to the end of this study period (33 weeks), but the decline in ZPP concentration lagged behind blood lead in reaching a plateau. These investigators logically concluded that the difficulty in demonstrating reliable blood lead-ZPP relationships may reflect differences in the time at which the two measures reach plateau. The authors also suggested that more reliance should therefore be placed on ZPP than on blood lead levels before permitting re-entry into areas of elevated lead exposure.

The threshold for the effect of lead on ZPP accumulation is affected by the relative spread of blood lead values and the corresponding concentrations of ZPP. In many cases these range from "normal" levels in nonexposed subjects to values reflecting considerable exposure. Furthermore, iron deficiency is also associated with ZPP elevation, particularly in children 2-3 years old or younger.

For EP elevation in adults, Roels et al. (1975b) found that the relationship of this effect to blood lead ended at 25-30 µg/dl, confirmed by the log-transformed data of Joselow and Flores (1977), Grandjean and Lintrup (1978), Odone et al. (1979), and Herber (1980). In children 10-15 years of age, the data of Roels et al. (1976) indicate an effect threshold of 15.5 µg/dl. In this study the threshold was taken as the point of intersection of two regression lines derived from two groups of children. The population dose-response relationship between EP and blood lead in these children indicated that EP levels were significantly higher (>2 standard deviations) than the reference mean in 50 percent of the children at a blood lead level of 25 µg/dl. In the age range of children studied here, iron deficiency is uncommon and these investigators did not note any significant hematocrit change in the exposure group. In fact, hematocrit was lower in the control group, although these subjects had lower ZPP levels. In this study, then, iron deficiency was unlikely to have been a confounding factor in the primary relationship. Piomelli et al. (1977) obtained a comparable threshold value (15.5 µg/dl) for lead's effect on ZPP elevation in children who were older than 4 years as well as those who were 2-4 years old. If iron deficiency was a factor in the results for this large study population (1816 children), one would expect a greater impact in the younger group, where the deficiency is more common.

Within the blood lead range considered "normal," i.e., below 30-40 µg/dl, assessment of any ZPP-blood lead relationship is strongly influenced by the relative analytical proficiency of the laboratory carrying out both measurements, particularly for blood lead at lower values.

The type of statistical treatment of the data is also a factor, as are some biological sources of variability. With respect to subject variability, Grandjean (1979) has documented that ZPP increases throughout adulthood, while hemoglobin remains relatively constant. Hence, age matching is a prerequisite. Similarly, the relative degree of ZPP response depends on gender: females show a greater response for a given blood lead level than do males (see discussion below).

Suga et al. (1981) claimed no apparent correlation between blood lead levels below 40 µg/dl and blood ZPP content in an adult population of 395 male and female subjects. The values for males and females were combined because of no measured differences in ZPP response, which is at odds with the studies of Stuik (1974), Roels et al. (1975b), Zielhuis et al. (1978a,b), Odone et al. (1979), and Toriumi and Kawai (1981). Also, EP was found to increase with increasing age, despite the fact that the finding of no correlation between blood lead and ZPP was based on a study population with all age groups combined.

Piomelli et al. (1982) investigated both the threshold for the effect of lead on EP accumulation and a dose-response relationship in 2004 children, 1852 of whom had blood lead values below 30 µg/dl. In this study, blood lead and EP measurements were done in facilities with a high proficiency for both blood lead and ZPP analyses. The study employed two statistical approaches (segmental line techniques and probit analysis), both of which revealed an average threshold blood lead level of 16.5 µg/dl in the full group and in the children with blood lead values below 30 µg/dl. In this report, the effect of iron deficiency and other non-lead factors was tested and removed using the Abbott formula (Abbott, 1925). With respect to population dose-response relationships, it was found that blood lead values of 28.6 and 35.2 µg/dl corresponded to significant EP elevation of more than 1 or 2 standard deviations, respectively, above a reference mean in 50 percent of the subjects. At a blood lead level of 30 µg/dl, furthermore, it was determined that 27 percent of children would have an EP greater than 53 µg/dl.

In a related study (Rabinowitz et al., 1986), simultaneous blood lead, ZPP, and hematocrit measurements were made semiannually on 232 normal infants during their first two years of life. The incidence of elevated ZPP (mean + 1 or 2 S.D.) was unrelated to blood lead below 15 µg/dl but was 4-fold greater above this threshold. The relationship persisted after correction for the small number (4 percent) of infants with a hematocrit below 33 percent. This survey extends the observations of Piomelli et al. (1982) to a younger and less lead-burdened population.

Comparison of EP elevations among adult males and females and children at a given blood lead level generally indicates that children and adult females are more sensitive to this effect of lead. Lamola et al. (1975a,b) demonstrated that the slope of ZPP versus blood lead was steeper in children than in adults. Roels et al. (1976) found that women and children were equally more sensitive in response than adult males, a finding also observed in the population studied by Odone et al. (1979). Other comparisons between adults, either as groups studied at random or in a voluntary lead exposure study, also document the sensitivity of females over males to this effect of lead (Stuik, 1974; Roels et al., 1975b, 1976, 1979; Toriumi and Kawai, 1981). The heightened response of females to lead-associated EP elevation has also been investigated in rats (Roels et al., 1978a) and has been shown to be related to hormonal interactions with lead, thus confirming the human data of Roels et al. (1975b, 1976, 1979) that iron status is not a factor in the phenomenon.

The effect of lead on iron incorporation into protoporphyrin in the heme biosynthetic pathway is not restricted to the erythropoietic system. Evidence of a generalized effect of lead on tissue heme synthesis at low levels of lead exposure comes from the recent studies of Rosen and coworkers (Rosen et al., 1980, 1981; Mahaffey et al., 1982) concerning lead-associated reductions in 1,25-dihydroxyvitamin D (1,25-$(OH)_2D$) (see Section 12.3.5). Such reductions probably occur because lead has an inhibitory effect on renal 1-hydroxylase, a heme-requiring cytochrome P-450 mediated mitochondrial enzyme system that converts 25-hydroxyvitamin D to 1,25-$(OH)_2D$. In an independent study, it has been shown in animals chronically exposed to moderate amounts of lead that kidney ferrochelatase activity is inhibited with elevation of EP, reducing the kidney heme pool for heme-requiring enzymes (Fowler et al., 1980). The low end of the blood lead range associated with lowered 1,25-$(OH)_2D$ levels and inhibited 1-hydroxylase activity corresponds to the level of lead associated with the onset of EP accumulation in erythropoietic tissue (see above). Sensitivity of renal mitochondrial 1-hydroxylase activity to lead is consistent with a large body of information showing the susceptibility of renal tubule cell mitochondria to injury by lead and with the chronic lead exposure animal model of Fowler et al. (1980), discussed in more detail below.

Formation of the heme-containing protein cytochrome P-450, which is an integral part of the hepatic mixed-function oxygenase system, has been documented in animals (Alvares et al., 1972; Scoppa et al., 1973; Chow and Cornish, 1978; Goldberg et al., 1978; Meredith and Moore, 1979) and humans (Alvares et al., 1975; Meredith et al., 1977; Fischbein et al., 1977; Saenger et al., 1984) as being affected by lead exposure, particularly acute lead intoxication. Many of these studies used altered drug detoxification rates as a functional measure of such effects. In the work of Goldberg et al. (1978), increasing the level of lead exposure in rats was correlated with both a steadily decreasing P-450 content of hepatic microsomes and decreased activity of the detoxifying enzymes aniline hydroxylase and aminopyrine demethylase. Similarly, the data of Meredith and Moore (1979) showed that continued dosing of rats with lead results in steadily decreased microsomal P-450 content, decreased total heme content of microsomes, and increased ALA-S activity.

Recently, Saenger and coworkers (1984) demonstrated that there was significantly reduced 6β-hydroxylation of cortisol in children having a positive ethylenediaminetetraacetic acid (EDTA) provocation test compared to a negative test group, under conditions of age matching and controlling for free cortisol. Because 6β-hydroxycortisol formation is mediated by hepatic cytochrome P-450 microsomal monooxygenase, lead appears to inhibit this system at relatively moderate levels of lead exposure in children.

According to Litman and Correia (1983), treatment of rats with either the organic porphyrinic agent 3,5-dicarbethoxy-2,6-dimethyl-4-ethyl-1,4-dihydropyridine (DDEP) or inorganic lead is associated with an inhibition of the hepatic enzyme system tryptophan pyrrolase, owing to depletion of the hepatic heme pool and resulting in elevated levels of tryptophan, serotonin, and 5-hydroxyindoleacetic acid in the brain. With infusion of heme, however, brain levels were restored to normal. These studies were carried out with phenobarbital induction of the enzyme system. The behavior of lead alone was not investigated.

Of interest in this regard are data relating to neural tissue. Studies of organotypic chick dorsal root ganglion in culture document that the nervous system has heme biosynthetic capability (Whetsell et al., 1978) and that this cell system elaborates decreased amounts of porphyrinic material in the presence of lead (Sassa et al., 1979). In a later investigation, Whetsell and coworkers (Whetsell and Kappas, 1981; Whetsell et al., 1984) reported that mouse dorsal root ganglion in culture exposed to lead for 6 weeks at 10^{-5} M (2 μg Pb/ml medium) showed progressive severe destruction of myelin and Schwann cells as well as alterations in axonal and neuronal ultrastructure. Because the co-administration of heme (10^{-4} M) prevented most of these destructive effects, particularly in Schwann cells, axons, and neurons, there is an indication of a relationship between inhibition by lead of heme biosynthesis in neural tissue and the morphological changes observed.

Chronic administration of lead to neonatal rats indicates that at low levels of exposure, with modest elevations of blood lead, there is a retarded growth in the respiratory chain hemoprotein cytochrome C and disturbed electron transport function in the developing rat cerebral cortex (Bull et al., 1979). The study of Holtzman et al. (1981) indicates that the cytochrome group affected and the brain region affected appear to differ with the age of the young animal at the start of dosing and the duration of dosing. All measured changes involved reduction at the $p < 0.05$ level. Young rats fed lead from birth for 3 weeks showed reduction in cytochrome aa_3 of cerebral mitochondria, while feeding for 4 weeks showed reduction in all cerebellar mitochondrial cytochromes. When feeding commenced at 2 weeks, the range of effects also depended on duration of exposure, with reduction of cytochrome b in cerebral mitochondria after one week, reduction in cytochrome $c + c_1$ in cerebral mitochondria after 2 weeks, and cerebellar cytochrome $c + c_1$ reduction after two weeks. These effects on the developing organism are accentuated by increased whole body lead retention in both developing children and experimental animals as well as by higher retention of lead in the brain of suckling rats as compared to adults.

Heme oxygenase activity is elevated in lead-intoxicated animals (Maines and Kappas, 1976; Meredith and Moore, 1979) in which relatively high dosing is employed. This indicates that normal repression of the enzyme's activity is lost, further adding to heme reduction and loss of regulatory control on the heme biosynthetic pathway.

The mechanism(s) underlying derangement of heme biosynthesis leading to ZPP accumulation in lead intoxication can be ascribed to impaired mitochondrial transport of iron, ferrochelatase inhibition, or a combination of both. Lead-induced effects on mitochondrial morphology and function, which are well known (Goyer and Rhyne, 1973; Fowler, 1978), may include impaired iron transport (Borová et al., 1973). Moreover, the resemblance of lead-associated ZPP accumulation to a similar effect of iron deficiency is consistent with the unavailability of iron to ferrochelatase rather than with direct enzyme inhibition. However, the porphyrin pattern seen in the congenital disorder erythropoietic porphyria, where ferrochelatase itself is affected, is different from that seen in lead intoxication.

Several animal studies indicate that the effects of lead on heme formation may involve both ferrochelatase inhibition and impaired mitochondrial transport of iron. Hart et al. (1980) observed that acute lead exposure in rabbits is associated with a two-stage hematopoietic response: an earlier phase that results in significant formation of free versus zinc protoporphyrin with considerable hemolysis and a later phase (where ZPP is formed) that otherwise resembles the common features of lead intoxication. Subacute exposure shows more of the typical porphyrin response reported with lead. These data may suggest that acute lead poisoning is quite different from chronic exposure in terms of the nature of hematological derangement.

Fowler et al. (1980) maintained rats on a regimen of oral lead, starting with exposure of their dams to lead in water and continuing through 9 months after birth at levels up to 250 ppm lead. The authors observed that the activity of kidney mitochondrial ALA-S and ferrochelatase, but not that of the cytosolic enzyme ALA-D, was inhibited. Ferrochelatase activity was inhibited at 25-, 50-, and 250-ppm exposure levels; activity was 63 percent of the control values at the 250-ppm level. Depression of state-3 respiration control ratios was observed for both succinate and pyruvate. Ultrastructurally, the mitochondria were swollen and lysosomes were rich in iron. In this study, reduced ferrochelatase activity was observed in association with mitochondrial injury and disturbance of function. The accumulation of iron may have been the result of phagocytized dead mitochondria or it may have represented intracellular accumulation of iron, owing to the inability of mitochondria to use the element.

Ibrahim et al. (1979) have shown that excess intracellular iron under conditions of iron overload is stored in cytoplasmic lysosomes. The observation of disturbed mitochondrial respiration suggests, as do the mitochondrial function data of Holtzman and Shen Hsu (1976) and Bull et al. (1979) for the developing nervous system, that intramitochondrial transport of iron would be impaired. Flatmark and Romslo (1975) demonstrated that iron transport in mitochondria is energy linked and requires an intact respiration chain at the level of cytochrome C, by which iron (III) on the C-side of the mitochondrial inner membrane is reduced before it is transported to the M-side and utilized in heme formation.

The above results are particularly interesting in terms of the relative responses of different tissues. While the kidney was affected, there was no change in blood indices of hematological derangement in terms of inhibited ALA-D activity or accumulation of ZPP. This suggests that there is a difference in dose-effect functions among different tissues, particularly with lead exposure during development of the organism. It appears that while blood indicators of erythropoietic effects of lead may be more accessible, they may not be the most sensitive indicators of heme biosynthesis derangement in other organs.

12.3.1.4 Effects of Lead on Coproporphyrin. An increased excretion of coproporphyrin in the urine of lead workers and children with lead poisoning has long been recognized, and urinary coproporphyrin measurement has been used as an indicator of lead poisoning. The mechanism of this enhanced production of coproporphyrin may be direct enzyme inhibition, accumulation of substrate secondary to inhibition of heme formation, or impaired intramitochondrial movement of the coproporphyrin. Excess coproporphyrin excretion differs from EP accumulation as an indicator of lead exposure. The former is a measure of ongoing lead intoxication without the lag in response seen with EP (Piomelli and Graziano, 1980).

In experimental lead intoxication, there is an accumulation of porphobilinogen and elevated excretion in urine, owing to inhibition by lead of the enzyme uroporphyrinogen (URO)-I-synthetase (Piper and Tephly, 1974). *In vitro* studies of Piper and Tephly (1974) using rat and human erythrocyte and liver preparations indicate that it is the erythrocyte enzyme URO-I-synthetase in both rats and humans that is sensitive to the inhibitory effect of lead; activity of the hepatic enzyme is relatively insensitive. Significant inhibition of the enzyme's activity occurs at 5 μM lead and virtually total inhibition of activity occurs in human erythrocyte hemolysates at 10^{-4} M. According to Piper and van Lier (1977), the lower sensitivity of hepatic URO-I-synthetase activity to lead is due to a protective effect afforded by a pteridine derivative, pteroylpolyglutamate. It appears that the protection does not occur through lead chelation, since hepatic ALA-D activity was reduced in the presence of lead. The studies of Piper and Tephly (1974) indicate that it is inhibition of URO-I-synthetase in erythroid tissue or erythrocytes that accounts for the accumulation of its substrate, porphobilinogen.

Unlike the case for experimental animals, accumulation of porphobilinogen in plasma and urine of lead-exposed humans has not been conclusively documented. Absence of porphobilinogen in urine is a differentiating characteristic in heme biosynthesis disturbance by lead versus the hepatic porphyrias, acute intermittent porphyrin, and variegate porphyria (Eubanks et al., 1983).

12.3.2 Effects of Lead on Erythropoiesis and Erythrocyte Physiology

12.3.2.1 Effects of Lead on Hemoglobin Production. Anemia is a manifestation (sometimes an early one) of chronic lead intoxication. Typically, the anemia is mildly hypochromic and usually normocytic. It is associated with reticulocytosis, owing to shortened cell survival, and the irregular presence of basophilic stippling. Its genesis lies in both decreased hemoglobin production and an increased rate of erythrocyte destruction. Not only is anemia commonly seen in children with lead poisoning, but it appears to be more severe and frequent among those with severe lead intoxication (World Health Organization, 1977; National Academy of Sciences, 1972; Lin-Fu, 1973; Betts et al., 1973).

While the anemia associated with lead intoxication in children shows features of iron-deficiency anemia, there are differences in cases of severe intoxication. These differences include reticulocytosis, basophilic stippling, and a significantly lower total iron binding capacity (TIBC). These latter features suggest that iron-deficiency anemia in young children is exacerbated by lead. The reverse is also true.

In young children, iron deficiency occurs at a significant rate, based on national (Mahaffey and Michaelson, 1980) and regional (Owen and Lippman, 1977) surveys, and it is known to be correlated with increased lead absorption in humans (Yip et al., 1981; Chisolm, 1981; Watson et al., 1980; Szold, 1974; Watson et al., 1958) and animals (Hamilton, 1978; Barton et al., 1978; Mahaffey-Six and Goyer, 1972). Hence, prevalent iron deficiency can be seen to potentiate the effects of lead in reduction of hemoglobin both by increasing lead absorption and by exacerbating the degree of anemia. Also, in young children, there is a negative correlation between hemoglobin level and blood lead levels (Adebonojo, 1974; Rosen et al., 1974; Betts et al., 1973; Pueschel et al., 1972). These studies generally involved children under 6 years of age in whom iron deficiency may have been a factor.

In adults, a negative correlation was observed in several studies at blood lead values usually below 80 μg/dl (Grandjean, 1979; Lilis et al., 1978; Roels et al., 1975a; Wada et al., 1973), while several studies did not report any relationship below 80 μg/dl (Valentine et al., 1982; Roels et al., 1979; Ramirez-Cervantes et al., 1978). In adults, iron deficiency would be expected to play less of a role in this relationship; Lilis et al. (1978) reported that the significant correlation between lead in blood and hemoglobin level was observed in workers in whom serum iron and TIBC were indistinguishable from controls.

The blood lead threshold for effects on hemoglobin has not been conclusively established. In children, this value appears to be about 40 µg/dl (World Health Organization, 1977), which is somewhat lower than in adults (Adebonojo, 1974; Rosen et al., 1974; Betts et al., 1973; Pueschel et al., 1972). Tola et al. (1973) observed no effect of lead on new workers until the blood lead had risen to a value of 50 µg/dl after about 100 days. The regression analysis data of Grandjean (1979), Lilis et al. (1978), and Wada et al. (1973) show persistence of the negative correlation of hemoglobin and blood lead below 50 µg/dl. Human population dose-response data for the lead-hemoglobin relationship are limited. Baker et al. (1979) calculated the following percentages of lead workers having a hemoglobin level of <14.0 g/dl blood at the specified blood lead concentrations: 5 percent at blood lead values of 40-59 µg/dl; 14 percent at blood lead values of 60-79 µg/dl; and 36 percent at blood lead values above 80 µg/dl. In 202 lead workers, Grandjean (1979) noted the following percentages of workers having a hemoglobin level below 14.4 g/dl at the specified blood lead concentrations: 17 percent at <25 µg/dl; 26 percent at 25-60 µg/dl; and 45 percent at >60 µg/dl.

The underlying mechanisms of lead-associated anemia appear to be a combination of reduced hemoglobin production and shortened erythrocyte survival because of direct cell damage. Under hemoglobin production, biosynthesis of globin, the protein moiety of hemoglobin, appears to be inhibited as a result of lead exposure (Dresner et al., 1982; Wada et al., 1972; White and Harvey, 1972; Kassenaar et al., 1957). In the study of White and Harvey (1972), two children treated for lead intoxication were studied for reticulocyte incorporation of a labeled amino acid into alpha and beta globin chains over a post-treatment period when blood lead was declining. These workers observed that a lag in *de novo* biosynthesis of alpha versus beta chains diminished toward a normal ratio (1.0) as blood lead approached 20 µg/dl. These data are in accord with the observation of Dresner et al. (1982), who noted a reduced globin synthesis (76 percent of controls) in rat bone marrow suspensions exposed to 1.0 µM lead.

Disturbance of globin biosynthesis is a consequence of lead's effects on heme formation because cellular heme regulates protein synthesis in erythroid cells (Levere and Granick, 1967) and regulates the translation of globin messenger RNA, which may also reflect the effect of lead on pyrimidine metabolism (Freedman and Rosman, 1976).

12.3.2.2 *Effects of Lead on Erythrocyte Morphology and Survival*. It is clear that there is a hemolytic component to lead-induced anemia in humans owing to shortened erythrocyte survival; the various aspects of this effect have been reviewed by Waldron (1966), Goldberg (1968), Moore et al. (1980), Valentine and Paglia (1980), and Angle and McIntire (1982).

The relevant studies of shortened cell life with lead intoxication include observations of the response of erythrocytes to mechanical and osmotic stress under *in vivo* and *in vitro* conditions. Waldron (1966) has discussed the frequent reports of increased mechanical fragility of erythrocytes from lead-poisoned workers, beginning with the observations of Aub et al. (1926). Increased osmotic resistance of erythrocytes from subjects with lead intoxication is a parallel finding, both *in vivo* (Aub and Reznikoff, 1924; Harris and Greenberg, 1954; Horiguchi et al., 1974) and *in vitro* (Qazi et al., 1972; Waldron, 1964; Clarkson and Kench, 1956). Using an apparatus called a coil planet centrifuge, Karai et al. (1981) studied erythrocytes of lead workers and found significant increases in osmotic resistance; at the same time, mean corpuscular volume and reticulocyte counts were not different from controls. The authors suggested that one mechanism of increased resistance involves impairment of hepatic lecithin-cholesterol acyltransferase, leading to a build-up of cholesterol in the cell membrane. This resembles the increased osmotic resistance seen in obstructive jaundice in which increased membrane cholesterol has been observed (Cooper et al., 1975). Karai et al. (1981) also reported an increased cholesterol-phospholipid ratio in lead workers' erythrocytes.

Fukumoto and coworkers (1983) studied the electrophoretic profiles of erythrocyte membrane proteins of a group of lead workers and found that compared to controls there was a significant negative correlation ($r = -0.51$, $p < 0.01$) between blood lead and a membrane transfer protein associated with Na^+ and water transport. It appears that one factor in reduced erythrocyte membrane permeability with lead exposure is a decrease in this protein.

Erythrokinetic data in lead workers and children with lead-associated anemia have been reported. Shortening of erythrocyte survival has been demonstrated by Hernberg et al. (1967a) using tritium-labeled difluorophosphonate. Berk et al. (1970) used detailed isotope studies of a subject with severe lead intoxication to determine shorter erythrocyte life span, while Leikin and Eng (1963) observed shortened cell survival in three of seven children. These studies, as well as the reports of Landaw et al. (1973), White and Harvey (1972), Albahary (1972), and Dagg et al. (1965), indicate that hemolysis is not the exclusive mechanism of anemia and that diminished erythrocyte production also plays a role.

The molecular basis for increased cell destruction with lead exposure includes the inhibition by lead of the activities of the enzymes (Na^+, K^+)-ATPase and pyrimidine-5'-nucleotidase (Py-5-N). Erythrocyte membrane (Na^+, K^+)-ATPase is a sulfhydryl enzyme and inhibition of its activity by lead has been well documented (Raghavan et al., 1981; Secchi et al., 1968; Hasan et al., 1967; Hernberg et al., 1967b). In the study of Raghavan et al. (1981), enzyme activity was inversely correlated with membrane lead content ($p < 0.001$) in lead workers with or without symptoms of overt lead toxicity, while correlation with whole blood lead was poor. With enzyme inhibition, there is irreversible loss of potassium ion from the cell with undisturbed input of sodium into the cell, resulting in a relative increase in sodium. Because the cells "shrink," there is a net increase in sodium concentration, which likely results in increased mechanical fragility and cell lysis (Moore et al., 1980).

In lead-exposed persons as well as in persons with a genetic deficiency of the enzyme Py-5-N, reduced activity leads to impaired phosphorolysis of the nucleotides cytidine and uridine phosphate, which are then retained in the cell and cause interference with the conservation of the purine nucleotides necessary for cellular energetics (Angle and McIntire, 1982; Valentine and Paglia, 1980). A more detailed discussion of lead's interaction with this enzyme is presented in Section 12.3.2.3.

In a series of experiments dealing with the hemolytic relationship of lead and vitamin E deficiency in animals, Levander et al. (1980) observed that lead exposure exacerbates the experimental hemolytic anemia associated with vitamin E deficiency by enhancing mechanical fragility, i.e., by reducing cell deformability. These workers note that vitamin E deficiency is seen with children having elevated blood lead levels, especially subjects having glucose-6-phosphate dehydrogenase (G-6-PD) deficiency, indicating that the synergistic relationship seen in animals may also exist in humans.

Glutathione is a necessary factor in erythrocyte function and structure. In workers exposed to lead, Roels et al. (1975a) found that there is a moderate but significant decrease in erythrocyte glutathione compared with controls. This appears to reflect lead-induced impairment of glutathione synthesis.

12.3.2.3 Effects of Lead on Pyrimidine-5'-Nucleotidase Activity and Erythropoietic Pyrimidine Metabolism. The presence in lead intoxication of basophilic stippling and an anemia of hemolytic nature is similar to what is seen in subjects having a genetically transmitted deficiency of Py-5-N, an enzyme mediating the phosphorolysis of the pyrimidine nucleotides, cytidine and uridine phosphates. With inhibition, these nucleotides accumulate in the erythrocyte or reticulocyte, ribonuclease-mediated ribosomal RNA catabolism is retarded in maturing cells, and the resulting accumulation of aggregates of incompletely degraded ribosomal fragments accounts for the phenomenon of basophilic stippling.

In characterizing the enzyme Py-5-N, Paglia and Valentine (1975) observed that its activity was particularly sensitive to inhibition by certain metals, particularly lead, prompting further investigation of the interplay between lead intoxication and disturbances of erythropoietic pyrimidine metabolism. Paglia et al. (1975) observed that in subjects occupationally exposed to lead but having no evidence of basophilic stippling or significant frequency of anemia, Py-5-N activity was reduced to about 50 percent of control subjects and was most impaired (about 11 percent of normal) in one worker with anemia. There was a general inverse correlation between enzyme activity and blood lead level. In this report, normal erythrocytes incubated with varying levels of lead showed detectable inhibition at levels as low as 0.1-1.0 μM and showed consistent 50 percent inhibition at about 10 μM lead. Subsequently, Valentine et al. (1976) observed that an individual with severe lead intoxication had an 85 percent decrease in Py-5-N activity, basophilic stippling, and accumulation of pyrimidine nucleotides, mainly cytidine triphosphate. Because these parameters approached values seen in the congenital deficiency of Py-5-N, the data suggest a common etiology for the hemolytic anemia and stippling in both lead poisoning and the congenital disorder.

Several other reports of investigations of Py-5-N activity and pyrimidine nucleotide levels in lead workers have been published (Paglia et al., 1977; Buc and Kaplan, 1978). In nine workers having overt lead intoxication and blood lead values of 80-160 μg/dl, Py-5-N activity was significantly inhibited, and the pyrimidine nucleotides constituted 70-80 percent of the total nucleotide pool, in contrast to trace levels in unexposed individuals (Paglia et al., 1977). In the study of Buc and Kaplan (1978), lead workers with or without overt lead intoxication all showed reduced activity of Py-5-N, which was inversely correlated with blood lead when the activity was expressed as a ratio with G-6-PD activity to accommodate an enhanced population of young cells due to hemolytic anemia. Enzyme inhibition was observed even when other indicators of lead exposure were negative.

Angle and McIntire (1978) observed that in 21 children 2-5 years old with blood lead levels of 7-80 μg/dl there was a negative linear correlation between Py-5-N activity and blood lead ($r = -0.60$, $p < 0.01$). Basophilic stippling was only seen in the child with the highest blood lead value and only two subjects had reticulocytosis. While adults tended to show a threshold for inhibition of Py-5-N at a blood lead level of 44 μg/dl or higher, there was no clear response threshold in these children. In a related investigation with 42 children 1-5 years old with blood lead levels of <10-72 μg/dl, Angle et al. (1982) noted the following: (1) an inverse correlation ($r = -0.64$, $p < 0.001$) between the logarithm of Py-5-N activity and blood lead; (2) a positive log-log correlation between cytidine phosphates and blood lead in 15 of these children ($r = 0.89$, $p < 0.001$); and (3) an inverse relationship in 12 subjects between the logarithm of enzyme activity and cytidine phosphates ($r = -0.796$, $p < 0.001$). Study of the various relationships at low levels was made possible by the use of anion-exchange high performance liquid chromatography. In these studies, there was no threshold of effects of lead on enzyme activity or cell nucleotide content even below 10 μg/dl. Finally, there was a significant positive correlation of pyrimidine nucleotide accumulation and the accumulation of ZPP.

In subjects undergoing therapeutic chelation with EDTA, Py-5-N activity increased, while there was no effect on pyrimidine nucleotides (Swanson et al., 1982). This indicates that the pyrimidine accumulation was associated with the reticulocyte.

The metabolic significance of Py-5-N activity inhibition and nucleotide accumulation with lead exposure is that they affect erythrocyte membrane stability and survival by alteration of cellular energetics (Angle and McIntire, 1982). In addition to cell lysis, feedback inhibition of mRNA and protein synthesis may result through the alteration of globin mRNA or globin chain synthesis by denatured mRNA. It was noted earlier that disturbances in heme production also affect the translation of globin mRNA (Freedman and Rosman, 1976). Hence, these two lead-associated disturbances of erythroid tissue function potentiate the effects of each other.

12.3.3 Effects of Alkyl Lead on Heme Synthesis and Erythropoiesis

In the discussion of alkyl lead metabolism in Chapter 10, Section 10.7, it was noted that transformations of tetraethyl and tetramethyl lead *in vivo* result in the generation not only of neurotoxic trialkyl lead metabolites but also products of further dealkylation, including inorganic lead. One would therefore expect alkyl lead exposure to be associated with, in addition to other effects, some of those effects classically related to inorganic lead exposure.

Chronic gasoline sniffing has been recognized as a problem habit among children in rural or remote areas (Boeckx et al., 1977; Kaufman, 1973). When such practice involves leaded gasoline, the potential exists for lead intoxication. Boeckx et al. (1977) conducted surveys of children in remote Canadian communities for the prevalence of gasoline sniffing and indications of chronic lead exposure. In one group of 43 children who all sniffed gasoline, mean ALA-D activity was only 30 percent that of control subjects; there was a significant correlation between the decrease in enzyme activity and the frequency of sniffing. A second survey of 50 children revealed similar results. Two children having acute lead intoxication associated with gasoline sniffing showed markedly lowered hemoglobin, elevated urinary ALA, and elevated urinary coproporphyrin. The authors estimated that more than half of the disadvantaged children residing in rural or remote areas of Canada may have chronic lead exposure via this habit; this estimate is consistent with the estimate of Kaufman (1973) of 62 percent for children in rural American Indian communities in the Southwest.

Robinson (1978) described two cases of pediatric lead poisoning due to habitual gasoline sniffing, one of which showed basophilic stippling. Hansen and Sharp (1978) reported that a young adult with acute lead poisioning due to chronic gasoline sniffing not only had basophilic stippling, but a sixfold increase in urinary ALA, elevated urinary coproporphyrin, and an EP level about fourfold above normal. In the reports of Boeckx et al. (1977) and Robinson (1978), increased lead levels measured in urine in the course of chelation therapy indicated that significant amounts of inorganic lead were present.

12.3.4 The Interrelationship of Lead Effects on Heme Synthesis and the Nervous System

Lead-associated disturbances in heme biosynthesis have been studied as a possible factor in the neurological effects of lead because of (1) the recognized similarity between classic signs of lead neurotoxicity and many, but not all, of the neurological components of the genetically transmitted (autosomal dominant) disorder acute intermittent porphyria, and (2) some unusual aspects of lead neurotoxicity. Both acute porphyria and lead intoxication with neurological symptoms are variably accompanied by abdominal pain, constipation, vomiting, paralysis or paresis, demyelination, and psychiatric disturbances (Dagg et al., 1965; Moore et al., 1980; Silbergeld and Lamon, 1980). According to Angle and McIntire (1982), some of the unusual features of lead neurotoxicity are consistent with deranged hematopoiesis: (1) a lag in production of neurological symptoms; (2) the incongruity of early deficits in affective and cognitive function with the regional distribution of lead in the brain; and (3) a better correlation of neurobehavioral deficits with erythrocyte protoporphyrin than with blood lead. The third feature, it should be noted, is not universally the case (Hammond et al., 1980; Spivey et al., 1979).

Available evidence points to three specific connections between the heme biosynthetic pathway and the nervous system in terms of the neurotoxic effects of lead: (1) the potential neurotoxicity of the heme precursor, ALA; (2) heme deficiency in tissues external to the nervous system, notably the liver; and (3) *in situ* impairment of heme availability in the nervous system.

While the nature and pattern of the derangements in heme biosynthesis in acute porphyria and lead intoxication differ in many respects, both involve excessive systemic accumulation and excretion of ALA, and this common feature has stimulated numerous studies of a connection between hemato- and neurotoxicity. *In vitro* data (Whetsell et al., 1978) have shown that the nervous system is capable of heme biosynthesis in the chick dorsal root ganglion. Sassa et al. (1979) found that the presence of lead in these preparations increases production of porphyrinic material, i.e., there is disturbed heme biosynthesis with accumulation of one or more porphyrins and, possibly, ALA. Millar et al. (1970) reported inhibited brain ALA-D activity in suckling rats exposed to lead, while Silbergeld et al. (1982) observed similar inhibition in brains of adult rats acutely exposed to lead. In the latter study, chronic lead exposure was also associated with a moderate increase in brain ALA without inhibition of ALA-D, suggesting an extra-neural source of the heme precursor. Finally, Dieter and Finley (1979) showed marked ALA-D activity depression in brain regions of avian subjects. Moore and Meredith (1976) administered ALA to rats and observed that exogenous ALA can penetrate the blood-brain barrier. These reports suggest that ALA can either be generated *in situ* in the nervous system or can enter the nervous system from elsewhere.

Neurochemical investigations of ALA action in the nervous system have evaluated interactions with the neurotransmitter gamma-aminobutyric acid (GABA). Interference with GABAergic function by exposure to lead is compatible with such clinical and experimental signs of lead neurotoxicity as excitability, hyperactivity, hyperreactivity, and, in severe lead intoxication, convulsions (Silbergeld and Lamon, 1980). Of particular interest is the similarity in chemical structures of ALA and GABA; these structures differ only in that ALA has a carbonyl group on the alpha carbon and GABA has a carbonyl group on the beta carbon.

While chronic lead exposure appears to alter neural pathways involving GABA function (Silbergeld et al., 1979), this effect cannot be duplicated in vitro using various levels of lead (Silbergeld et al., 1980a). This suggests that lead does not impart the effect by direct interaction or that an intact multi-pathway system is required. In vitro studies (Silbergeld et al., 1980a; Nicoll, 1976) demonstrate that ALA can displace GABA from synaptosomal membranes associated with synaptic function of the neurotransmitter on the GABA receptor, but that it is less potent than GABA by a factor of 10^3-10^4, suggesting that levels of ALA achieved even with severe intoxication may not be effectively competitive.

A more significant role for ALA in lead neurotoxicity may well be related to the observation that GABA release is subject to negative feedback control through presynaptic receptors on GABAergic terminals (Snodgrass, 1978; Mitchell and Martin, 1978). Brennan and Cantrill (1979) found that ALA inhibits K^+-stimulated release of GABA from preloaded synaptosomes by functioning as an agonist at the presynaptic receptors. The effect is evident at 1.0 μM ALA, and it is abolished by the GABA antagonists bicuculline and picrotoxin. Of interest also is the demonstration (Silbergeld et al., 1980a) that synaptosomal release of preloaded ^{3}H-GABA, both resting and K^+-stimulated, is also inhibited in animals chronically treated with lead, paralleling the in vitro data of Brennan and Cantrill (1979) using ALA.

Silbergeld et al. (1982) described the comparative effects of lead and the agent succinylacetone, given acutely or chronically to adult rats, in terms of disturbances in heme synthesis and neurochemical indices. Succinylacetone, a metabolite that can be isolated from the urine of patients with hereditary tyrosinemia (Lindblad et al., 1977), is a potent inhibitor of heme synthesis, exerting its effect by ALA-D inhibition and derepression of ALA synthetase (Tschudy et al., 1980, 1981). In vivo, both agents showed significant inhibition of high affinity Na^+-dependent uptake of ^{14}C-GABA by cortex, caudate, and substantia nigra. However, neither agent affected GABA uptake in vitro. Similarly, both chronic and acute lead treatment and chronically administered succinylacetone reduced the seizure threshold to the GABA antagonist, picrotoxin. While these agents may involve entirely different mechanisms of toxicity to the GABAergic pathway, the fact remains that two distinct potent inhibitors of the heme biosynthetic pathway and ALA-D, which do not impart a common neurochemical effect by direct action on a neurotransmitter function, have a common neurochemical action in vivo.

Several important studies in experimental systems strongly indicate that the key factor in the connection between heme biosynthesis and neurotoxicity may very well be a reduction in the levels of heme itself, rather than behavior of its precursor, ALA. Badawy (1978) first described the role of tryptophan pyrrolase in the relationship of heme biosynthesis and neurotoxic manifestations of the hepatic porphyrias. Using a porphyric rat model, Litman and Correia (1983) have reported that lead and the porphyrinic agent DDEP are both associated with inhibition of the hepatic heme-requiring enzyme system, tryptophan pyrrolase, via reduction of the free hepatic heme pool. This results not only in elevated plasma tryptophan but also in significantly elevated brain levels of tryptophan, serotonin, and 5-hydroxyindoleacetic acid. Of particular interest is the effect of subsequent infusion of heme, which reduced the elevated levels of these substances to normal amounts. Since, as noted by the authors, heme does not penetrate the blood-brain barrier, heme repletion had its effect in the liver. This was confirmed by increases in both hepatic heme content and enzyme activity after heme infusion. These data are relevant to some of the common features of acute porphyria and disturbances in tryptophan metabolism noted by Litman and Correia (1983):

(1) Elevated tryptophan levels have been associated with human hepatic encephalopathy.

(2) Elevation in circulating tryptophan in rats produces structural alterations of brain astrocytes, oligodendroglia, and neurons, as well as Purkinje cell degeneration and axonal wasting. These neurohistological changes resemble those seen in victims of acute porphyria attacks.

(3) The pharmacological effects of serotonin in the central nervous system resemble the neurological manifestations of acute porphyria attacks.

(4) Administration of tryptophan and serotonin to humans yields symptoms greatly overlapping those of acute porphyria attacks: psychomotor disturbances, abdominal pain, nausea, and dysuria.

(5) Porphyric subjects show abnormal tryptophan metabolism and urinary excretion of large amounts of 5-hydroxyindoleacetic acid.

In the above study, phenobarbital induction of the enzyme system was employed. The behavior of lead alone has not been investigated. In studies related to heme reduction in the nervous system itself, Whetsell and Kappas (1981) and Whetsell et al. (1984) showed that co-administration of heme and lead prevented most of the neuropathic responses to lead in cultured mouse dorsal root ganglion seen with lead alone (see Section 12.3.13). These results strongly suggest that reduced heme levels in the neural tissue due to the presence of lead are associated with the adverse effects observed. Because this tissue culture system is known to carry out heme biosynthesis (Whetsell et al., 1978; Sassa et al., 1979), it is highly likely that lead impairs neural heme biosynthesis.

Human data relating the hemato- and neurotoxicity of lead are limited. Hammond et al. (1980) reported that the best correlates of the frequency of neurological symptoms in 28 lead workers were urinary and plasma ALA, as well as blood lead levels, both of which showed a higher correlation than EP. These data support a connection between heme biosynthesis impairment and neurological effects of ALA. Of interest here is the clinical report of Lamon et al. (1979) describing the effect of hematin [Fe(III)-heme] given parenterally to a subject with lead intoxication. Over the course of treatment (16 days), urinary coproporphyrin and ALA significantly dropped and neurological symptoms such as lower extremity numbness and aching diminished. Blood lead levels were not altered during the treatment. Although remission of symptoms in this subject may have been spontaneous, the outcome parallels that observed in hematin treatment of subjects with acute porphyria in similar reduction of heme indicators and relief of symptoms (Lamon et al., 1979).

Taken collectively, all of the available data suggest the following:

(1) Delta-aminolevulinic acid formed *in situ* or entering the brain may well be neurotoxic by impairing GABAergic function in particular. It inhibits K^+-stimulated GABA release by interaction with presynaptic receptors, where ALA appears to be particularly potent at very low levels (1.0 μm), based on *in vitro* results.

(2) Decreased levels of heme in the liver due to lead exposure inhibit the activity of tryptophan pyrrolase, resulting in elevations of tryptophan, serotonin, and 5-hydroxyindoleacetic acid in brain. Such increases are reversed by infusion of heme.

(3) Heme reduction in neural tissue, as a result of lead's effect on heme biosynthesis, is associated with tissue injury; such injury is prevented by heme co-administration.

12.3.5 Interference with Vitamin D Metabolism and Associated Physiological Processes

A new dimension to the human toxicology of lead is presented by lead's interaction with the vitamin D-endocrine system. Recent evidence of lead-induced disturbances in vitamin D metabolism in humans and animals, particularly with respect to lead-related reductions in the biosynthesis of the hormonal metabolite 1,25-dihydroxyvitamin D ($1,25\text{-}(OH)_2D$), are of special concern for two reasons: (1) $1,25\text{-}(OH)_2D$ appears to serve many more physiological roles than just mediation of calcium homeostasis and metabolic function, and (2) even moderate levels of lead exposure in children are associated with vitamin D disturbances that parallel certain genetic metabolic disorders and other disease states, as well as severe kidney dysfunction.

It appears likely that lead-induced reductions in heme underlie the effects seen in the vitamin D-endocrine system. This origin would account for the similarities in "thresholds" for the effects of lead on both erythrocyte protoporphyrin accumulation and decreases in levels of 1,25-$(OH)_2D$. It also typifies a cascade of biological effects among various organ and physiological systems of the body, effects that can ultimately encompass the entire organism (this is graphically depicted in Chapter 13). Collectively, the interrelationships of calcium and lead metabolism as well as lead's effects on 1,25-$(OH)_2D$ provide one molecular and mechanistic basis for the classic observation by Aub et al. (1926) that "lead follows the calcium stream."

12.3.5.1 Relevant Clinical Studies. As initially reported by Rosen et al. (1980), lead intoxicated children with blood lead concentrations of 33-120 µg/dl have a marked reduction in serum levels of 1,25-$(OH)_2D$. The most striking decrease in circulating 1,25-$(OH)_2D$ levels was found in children whose blood lead levels were greater than 62 µg/dl. Nonetheless, highly significant and profound depressions in circulating 1,25-$(OH)_2D$ levels were found also in children whose blood lead concentrations ranged from 33 to 55 µg/dl. Children whose blood lead values were above 62 µg/dl also showed a significant decrease in serum total calcium and ionized calcium (Ca), while serum parathyroid hormone (PTH) concentrations were significantly elevated. Under these conditions, and in the face of decreased dietary intake of calcium, it is anticipated that the recognized modulators of 1,25-$(OH)_2D$ synthesis (PTH, Ca^{2+}, inorganic phosphorus [P_i]) would enhance production of the vitamin D hormone. Since there was in fact a reduction in circulating concentrations of 1,25-$(OH)_2D$, this suggests that production of the vitamin D hormone was actually impaired.

On the basis of significant negative correlations between blood levels of lead and serum levels of 1,25-$(OH)_2D$ and negative correlations between erythrocyte protoporphyrin and 1,25-$(OH)_2D$ in children with blood lead concentrations of 33-120 µg/dl, it is reasonable to conclude that the lead ion impairs the production of 1,25-$(OH)_2D_3$, as aluminum does in children undergoing total parenteral nutrition (Rosen and Chesney, 1983).

The 1-hydroxylation step to produce the vitamin D hormone is carried out in the mitochondria of the renal tubule by a complex cytochrome P-450 enzyme system (Rosen and Chesney, 1983). The ingredients of this enzyme system include intact mitochondria, Krebs cycle substrates, cytochrome P-450 electron transport, oxidative phosphorylation, and generation of NADPH (nicotinamide adenine dinucleotide phosphate, reduced). The biosynthesis of the vitamin D hormone is controlled in large part by the functional integrity of mitochondria, by the ionic (Ca, P_i) microenvironment of the extracellular fluid, and by the uptake of calcium by mitochondria (including the delicate homeostasis characteristic of intracellular calcium concentrations and calcium pumps). It is clear, therefore, based upon lead's toxic effects on mitochondria, cellular energetics, and cytochrome P-450 electron transport in several studies, including some on children (Saenger et al., 1984; Piomelli et al., 1982), that lead most likely impairs the 1-hydroxylase enzyme system, although altered peripheral metabolism of $1,25\text{-}(OH)_2D$ cannot be completely ruled out. As noted in Section 12.5, furthermore, lead inhibits renal ferrochelatase with accumulation of EP leading to a reduction in the kidney heme pool and reduced availability of heme for renal 1-hydroxylase (Fowler et al., 1980).

Lead's impairment of the 1-hydroxylase enzyme system in lead intoxicated children is strongly supported by additional information: 1) $1,25\text{-}(OH)_2D$ levels in serum returned to normal values within two days following chelation therapy, while no changes were found in 25-(OH)D levels, and 2) a strong negative correlation between $1,25\text{-}(OH)_2D$ values and blood lead was found over the entire range (12-120 µg/dl) of blood lead levels measured in the study (Rosen et al., 1980; Mahaffey et al., 1982). Furthermore, no change in the slope of the regression line between $1,25\text{-}(OH)_2D$ and blood lead was found for blood lead values above or below 30 µg/dl (Mahaffey et al., 1982). These findings provide considerable support for the view that lead interferes with normal ionic transport in cells and with the functional integrity of mitochondria that carry out this 1-hydroxylation. In terms of ionic transport, current information indicates that increasing extracellular and intracellular concentrations of calcium depress production of the vitamin D hormone (Rosen and Chesney, 1983). If renal tubule cells accumulate high concentrations of calcium after exposure to lead, as do hepatocytes (Pounds et al., 1982a, Pounds and Mittelstaedt, 1983), osteoclasts (Rosen, 1983, 1985), and brain cells (Silbergeld and Adler, 1978), renal tubule cells may consequently "turn off" $1,25\text{-}(OH)_2D$ production. Such an effect is likely to be reversible when lead is decreased in the extracellular fluid, as it is in children after therapy with $CaNa_2EDTA$.

In summary, lead's effect(s) on the complex 1-hydroxylase enzyme system may be expressed in one or several components of the enzyme (e.g., cellular energetics, integrity of mitochondria). Simultaneously, lead may interfere with ionic regulation of $1,25\text{-}(OH)_2D_3$ biosynthesis. The fact that such effects can be reversed, at least insofar as $1,25\text{-}(OH)_2D$ levels may recover to normal values after chelation therapy, does not suggest that these effects of lead are necessarily transient or subject to physiological adaptation. Thus far, reversibility has been known to occur only after medical intervention.

12.3.5.2 Experimental Studies. Smith et al. (1981) observed depressions of plasma $1,25\text{-}(OH)_2D$ in rats fed 0.82 percent lead as lead acetate. Moreover, lead ingestion totally blocked the intestinal calcium transport response to the vitamin D hormone. Though the dose of lead and the resulting blood lead concentrations were high in this study, it confirms the effect(s) of lead on vitamin D metabolism reported in children. A recent study demonstrated directly that renal production and tissue levels of the vitamin D hormone were reduced in a dose-related fashion in chicks fed a diet supplemented with lead (Edelstein et al., 1984). Previous studies have shown that vitamin D and $1,25\text{-}(OH)_2D_3$ enhance lead acetate absorption in the distal small intestine of the rat, whereas vitamin D-dependent calcium absorption occurs in the proximal duodenum (Smith et al., 1978; Mahaffey et al., 1979). It is likely that vitamin D affects lead absorption in a manner somewhat different from the manner in which it affects calcium absorption (Smith et al., 1978; Mykkänen and Wasserman, 1982). It is of interest that high doses of vitamin D and 1,25-$(OH)_2D_3$ do not markedly increase lead absorption above that achieved with physiological doses (Smith et al., 1978).

12.3.5.3 Implications of Lead Effects on Vitamin D Metabolism

12.3.5.3.1 Direct Metabolic Consequences of Vitamin D Metabolism Interference in Children. A lower daily intake of calcium, as observed in lead intoxicated children (Sorrell et al., 1977; Rosen et al., 1980), accompanied by a relative decrease in 1,25-$(OH)_2D$-stimulated formation of calcium-binding protein (CaBP), may permit lead to compete favorably with calcium for mucosal proteins and similar absorption sites in the intestine. In addition, experimental study with animal CaBP has demonstrated a much greater affinity of this intestinal protein for lead than for calcium (Fullmer et al., 1985). Such findings help explain the negative correlations found between calcium intake and blood lead and between serum calcium and blood lead values (Sorrell et al., 1977).

Furthermore, depression in serum ionized calcium during lead intoxication (Rosen et al., 1980) may enhance the movement of lead from hard tissue to critical organ sites in soft tissues. In bone organ culture, decreasing the concentration of calcium in the medium enhances mobilization of previously incorporated radioactive lead from bone explants to the medium (Rosen and Markowitz, 1980). Among other things, these findings indicate that reduced 1,25-$(OH)_2D$ levels do not serve to "protect" soft target organs such as brain and kidney from lead deposition by sequestering the metal in bone. Further, empirical support for this conclusion may be found in the results of Smith et al. (1981), who reported no consistent differences in rats' kidney lead content as a function of the presence or absence of a vitamin D supplement in the diet. These investigators also found no significant differences in the blood lead concentrations of the rats as a function of vitamin D supplementation. In summary, there is little reason to suppose that reduced levels of 1,25-$(OH)_2D$ might function as part of a negative feedback process to reduce further absorption of lead or to mitigate its toxic effects on various target organs.

12.3.5.3.2 Other Childhood Diseases Associated with a Reduction in Circulating 1,25-$(OH)_2D$ as a Reflection of Depressed Biosynthesis. At blood lead levels of 33-55 μg/dl (Rosen et al., 1980), 1,25-$(OH)_2D$ levels are reduced to levels comparable to those observed in children who have severe renal insufficiency with loss of about two-thirds of their normal renal function (Rosen and Chesney, 1983; Chesney et al., 1983). Also, at blood lead levels of 33-120 μg/dl, analogous depressions in 1,25-$(OH)_2D$ concentrations ($\leq$20 pg/ml) are found in:

(1) Vitamin D-dependent rickets, type I--an inborn error of vitamin D metabolism in which the 1-hydroxylase enzyme system (or a component thereof) is virtually absent;

(2) Oxalosis--an inborne error of metabolism in which calcium oxalate crystals are deposited throughout the body, including the kidney, and result in chronic renal insufficiency;

(3) Hormone-deficient hypoparathyroidism--thought to be an autoimmune disorder, hereditary in some cases, that is characterized by parathyroid hormone deficiency and, as a result, decreased production of the vitamin D hormone;

(4) Aluminum intoxication in children undergoing total parenteral nutrition--has occurred when the casein hydrolysates used were contaminated with aluminum.

These disorders are reviewed by Rosen and Chesney (1983) and Chesney et al. (1983).

12.3.5.3.3 Physiological Functions of 1,25-$(OH)_2D_3$ at the Cellular Level. The vitamin D-endocrine system is responsible in large part for the maintenance of extra- and intracellular calcium homeostasis (Rasmussen and Waisman, 1983; Wong, 1983; Shlossman et al., 1982; Rosen and Chesney, 1983). As a result, the integrity of cells of diverse function is preserved, as are numerous calcium-mediated functions. It is known that calcium, an important participant in the hormonal responses of many target cell systems (Rasmussen and Waisman, 1983), acts not only as a second messenger, but also as a modulator of cyclic nucleotide metabolism. The temporal and spatial regulation of cellular calcium is exceedingly important in the response of a variety of cells to hormonal and electrical stimuli.

Lead alone (without hormones) produces an overamplification of calcium influx in hepatocytes (Pounds et al., 1982a), osteoclasts (Rosen, 1983, 1985), and brain slices (Silbergeld and Adler, 1978) at relatively low concentrations. As a result, calcium-mediated cell functions are perturbed (Pounds et al., 1982b). Based upon these findings, it is reasonable to conclude that modulation in cellular calcium metabolism induced by lead at relatively low concentrations may have the potential of disturbing multiple functions of different tissues that depend upon calcium as a second messenger. Perturbations in cellular calcium homeostasis may thereby result from the effects of lead alone; but these effects may be enhanced when coupled with decreased production of 1,25-$(OH)_2D_3$ and reduction in serum (and extracellular fluid) ionized calcium values observed in lead intoxicated children.

Calmodulin is of central importance as an intracellular calcium receptor protein. Its nearly universal distribution in mammalian cells emphasizes further that calcium serves as a "universal" second messenger. As such, calmodulin regulates several enzyme systems and transport processes. The list of calcium-sensitive reactions modulated by the calmodulin-calcium complex is rapidly expanding (see review by Cheung, 1980). Recently, it has been shown that lead can replace calcium in the activation of calmodulin-sensitive processes (Habermann et al., 1983), including potassium loss from erythrocytes (Goldstein and Ar, 1983). Though at an early stage of investigation, it is conceivable that a molecular model of lead toxicity may include (in addition to those processes cited above) intracellular occupation of calcium-binding sites on calmodulin. Since calmodulin regulates multiple cell activities, such a mechanism may underlie some of the diverse effects of lead. Lead alone and lead's interaction(s) with calmodulin and intracellular calcium homeostasis are inherently coupled to the vitamin D-endocrine system.

12.3.5.3.4 Cell Differentiation/Maturation. Tumor cell lines possess cytosol receptors to which 1,25-$(OH)_2D_3$ binds specifically (Tanaka et al., 1982; Shiina et al., 1983; Honma et al., 1983). Human promyelocytic leukemia cells (HL-60) can be induced to differentiate *in vitro* by 1,25-$(OH)_2D_3$. Differentiation-associated properties, such as phagocytosis and C3 rosette formation, were induced by as little as 0.12 nM 1,25-$(OH)_2D_3$. As cells exhibited differentiation, the viable cell number was decreased to less than half of the control (Tanaka et al., 1982). A specific cytosol protein that bound 1,25-$(OH)_2D_3$ was found in these HL-60 cells; its physical and biochemical properties closely resembled those found in "classical" vitamin D target tissues. These and other studies noted above indicated that 1,25-$(OH)_2D_3$ induced differentiation of HL-60 cells by a mechanism similar to that proposed for the classical concept of steroid hormone action. This common mechanism of steroid hormone action includes binding of hormone to a cytosol receptor (to form a hormone-receptor complex) and subsequent movement of this complex into the nucleus where it binds to chromatin.

A recent study by Honma et al. (1983) showed that the survival time of syngeneic SL mice inoculated with murine myeloid leukemia cells (ML) was markedly prolonged by 1-25-$(OH)_2D_3$ treatment (12.5-50 pmol per mouse). Evidence indicated that induction of differentiation of ML cells into macrophages *in vitro* was correlated with its effect in prolonging survival time; and it was suggested that the role of 1,25-$(OH)_2D_3$ in decreasing leukemogenicity of ML cells *in vivo* is due to its effect in suppressing proliferation and inducing differentiation of ML cells *in vitro* (Honma et al., 1983).

It is evident, therefore, that the differentiation in HL-60 cells (and other cell lines) caused by 1,25-$(OH)_2D_3$ is a manifestation of the normal action of this hormone to elicit maturation of myeloid stem cells into macrophages. Because macrophages are thought to be the precursor of bone resorbing osteoclasts, this is a logical mechanism whereby 1,25-$(OH)_2D_3$ brings about calcium resorption/homeostasis through recruitment of cells competent in bone remodeling. Moreover, parathyroid hormone is thought to act both directly to stimulate osteoclast production from myeloid precursors and on T-lymphocytes to cause the elaboration of putative osteoclast-enhancing factors. It appears, therefore, that the vitamin D hormone regulates calcium homeostasis and also participates directly in bone turnover by orchestrating the population of cells within bone.

12.3.5.3.5 Immunoregulatory Role of the Vitamin D Hormone. The widespread distribution of receptors for 1,25-$(OH)_2D_3$ in tissues not thought to play a role in mineral metabolism has made it clear that the vitamin D hormone plays a wider biologic role than was previously thought (Kadowaki and Norman, 1984a,b; Stumpf et al., 1982; Clark et al., 1981; Gelbard et al., 1980). Receptors for 1,25-$(OH)_2D_3$ are present in normal human monocytes and malignant lymphocytes (Provvedini et al., 1983; Bhalla et al., 1983). It has also been shown that macrophages from vitamin D-deficient mice have impaired phagocytic and inflammatory responses correctable by 1,25-$(OH)_2D_3$ repletion (Bar-Shavit et al., 1981).

T and B lymphocytes obtained from normal humans also expressed the 1,25-$(OH)_2D_3$ receptor after the lymphocytes had been activated by mitogenic lectins and Epstein-Barr virus (Provvedini et al., 1983; Bhalla et al., 1983). The mitogenic lectin phytohemagglutinin (PHA) stimulates T lymphocyte proliferation and induces the production of various lymphokines, including interleukin-2 (IL-2), which is important for the growth of T cells. Recently, it has been demonstrated that 1,25-$(OH)_2D_3$ (at picomolar concentrations) inhibits the growth-promoting lymphokine IL-2 and the proliferation of PHA-stimulated lymphocytes obtained from normal humans (Tsoukas et al., 1984). These results confirm and extend earlier evidence that 1,25-$(OH)_2D_3$ receptors are expressed in T lymphocytes activated with mitogenic lectins (Provvedini et al., 1983; Bhalla et al., 1983). In light of suggestions that calcium translocation is involved in the mitogen-induced activation of lymphocytes and in view of the well-recognized calcitropic effects of 1,25-$(OH)_2D_3$ on mineral-dependent target tissues, it may be that the suppressive effect of the vitamin D hormone on IL-2 is mediated by calcium translocation (Tsoukas et al., 1984). However mediated, this effect demonstrates the immunoregulatory role of 1,25-$(OH)_2D_3$ and, thus, another possible means by which lead could affect immunity (see Section 12.8).

12.3.6 Summary and Overview

12.3.6.1 Effects of Lead on Heme Biosynthesis. The effects of lead on heme biosynthesis are well known because of their clinical prominence and the numerous studies of such effects in humans and experimental animals. The process of heme biosynthesis starts with glycine and succinyl-coenzyme A, proceeds through formation of protoporphyrin IX, and culminates with the insertion of divalent iron into the porphyrin ring to form heme. In addition to being a constituent of hemoglobin, heme is the prosthetic group of many tissue hemoproteins having variable functions, such as myoglobin, the P-450 component of the mixed-function oxygenase system, and the cytochromes of cellular energetics. Hence, disturbance of heme biosynthesis by lead poses the potential for multiple-organ toxicity.

In investigations of lead's effects on the heme synthesis pathway, most attention has been devoted to the following: (1) stimulation of mitochondrial delta-aminolevulinic acid synthetase (ALA-S), which mediates formation of delta-aminolevulinic acid (ALA); (2) direct inhibition of the cytosolic enzyme, delta-aminolevulinic acid dehydrase (ALA-D), which catalyzes formation of porphobilinogen from two units of ALA; and (3) inhibition of insertion of iron (II) into protoporphyrin IX to form heme, a process mediated by ferrochelatase.

Increased ALA-S activity has been found in lead workers as well as in lead-exposed animals, although an actual decrease in enzyme activity has also been observed in several experimental studies using different exposure methods. It appears, then, that the effect on ALA-S activity may depend on the nature of the exposure. Using rat liver cells in culture, ALA-S activity was stimulated in vitro at lead levels as low as 5.0 μM or 1.0 μg/g preparation. The increased activity was due to biosynthesis of more enzyme. The blood lead threshold for stimulation of ALA-S activity in humans, based on a study using leukocytes from lead workers, appears to be about 40 μg/dl. Whether this apparent threshold applies to other tissues depends on how well the sensitivity of leukocyte mitochondria mirrors that in other systems. The relative impact of ALA-S activity stimulation on ALA accumulation at lower lead exposure levels appears to be much less than the effect of ALA-D activity inhibition. ALA-D activity is significantly depressed at 40 μg/dl blood lead, the point at which ALA-S activity only begins to be affected.

Erythrocyte ALA-D activity is very sensitive to inhibition by lead. This inhibition is reversed by reactivation of the sulfhydryl group with agents such as dithiothreitol, zinc, or zinc and glutathione. Zinc levels that achieve reactivation, however, are well above physiological levels. Although zinc appears to offset the inhibitory effects of lead observed in animal studies and in human erythrocytes in vitro, lead workers exposed to both zinc and lead do not show significant changes in the relationship of ALA-D activity to blood lead when compared with workers exposed just to lead. Nor does the range of physiological zinc levels in nonexposed subjects affect ALA-D activity. In contrast, zinc deficiency in animals significantly inhibits ALA-D activity, with concomitant accumulation of ALA in urine. Because zinc deficiency has also been demonstrated to increase lead absorption, the possibility exists for the following dual effects of such deficiency on ALA-D activity: (1) a direct effect on activity due to reduced zinc availability; and (2) increased lead absorption leading to further inhibition of activity.

Erythrocyte ALA-D activity appears to be inhibited at virtually all blood lead levels measured so far, and any threshold for this effect in either adults or children remains to be determined. A further measure of this enzyme's sensitivity to lead is a report that rat bone marrow suspensions show inhibition of ALA-D activity by lead at a level of 0.1 μg/g suspension. Inhibition of ALA-D activity in erythrocytes apparently reflects a similar effect in other tissues. Hepatic ALA-D activity in lead workers was inversely correlated with erythrocyte activity as well as blood lead levels. Of significance are experimental animal data showing that (1) brain ALA-D activity is inhibited with lead exposure, and (2) this inhibition appears to occur to a greater extent in developing animals than in adults, presumably reflecting greater retention of lead in developing animals. In the avian brain, cerebellar ALA-D activity is affected to a greater extent than that of the cerebrum and, relative to lead concentration, shows inhibition approaching that occurring in erythrocytes.

Inhibition of ALA-D activity by lead is reflected by elevated levels of its substrate, ALA, in blood, urine, and soft tissues. Urinary ALA is employed extensively as an indicator of excessive lead exposure in lead workers. The diagnostic value of this measurement in pediatric screening, however, is limited when only spot urine collection is done; more satisfactory data are obtainable with 24-hr collections. Numerous independent studies document a direct correlation between blood lead and the logarithm of urinary ALA in human adults and children; the blood lead threshold for increases in urinary ALA is commonly accepted as 40 µg/dl. However, several studies of lead workers indicate that the correlation between urinary ALA and blood lead continues below this value; one study found that the slope of the dose-effect curve in lead workers depends on the level of exposure.

The health significance of lead-inhibited ALA-D activity and accumulation of ALA at lower lead exposure levels is controversial. The "reserve capacity" of ALA-D activity is such that only the level of inhibition associated with marked accumulation of the enzyme's substrate, ALA, in accessible indicator media may be significant. However, it is not possible to quantify at lower levels of lead exposure the relationship of urinary ALA to target tissue levels or to relate the potential neurotoxicity of ALA at any accumulation level to levels in indicator media. Thus, the blood lead threshold for neurotoxicity of ALA may be different from that associated with increased urinary excretion of ALA.

Accumulation of protoporphyrin in erythrocytes of lead-intoxicated individuals has been recognized since the 1930s, but it has only recently been possible to quantitatively assess the nature of this effect via development of sensitive, specific microanalysis methods. Accumulation of protoporphyrin IX in erythrocytes results from impaired placement of iron (II) in the porphyrin moiety in heme formation, an intramitochondrial process mediated by ferrochelatase. In lead exposure, the porphyrin acquires a zinc ion in lieu of native iron, thus forming zinc protoporphyrin (ZPP), which is tightly bound in available heme pockets for the life of the erythrocytes. This tight sequestration contrasts with the relatively mobile nonmetal, or free, erythrocyte protoporphyrin (FEP) accumulated in the congenital disorder erythropoietic protoporphyria.

Elevation of erythrocyte ZPP has been extensively documented as exponentially correlated with blood lead in children and adult lead workers and is currently considered one of the best indicators of undue lead exposure. Accumulation of ZPP only occurs in erythrocytes formed during lead's presence in erythroid tissue; this results in a lag of at least several weeks before its buildup can be measured. The level of ZPP accumulation in erythrocytes of newly employed lead workers continues to increase after blood lead has already reached a plateau. This influences the relative correlation of ZPP and blood lead in workers with short exposure histories. Also, the ZPP level in blood declines much more slowly than blood lead, even after removal from exposure or after a drop in blood lead. ZPP level also appears to be a more reliable indicator of continuing intoxication from lead resorbed from bone in former lead workers long removed from heavy lead exposure.

The threshold for detection of lead-induced ZPP accumulation is affected by the relative spread of blood lead and corresponding ZPP values measured. In young children (<4 yr old), the ZPP elevation associated with iron-deficiency anemia must also be considered. In adults, numerous studies indicate that the blood lead threshold for ZPP elevation is about 25-30 μg/dl. In children 10-15 years old, the threshold is about 16 μg/dl; for this age group, iron deficiency is not a factor. In one study, children over 4 years old showed the same threshold, 15.5 μg/dl, as a second group under 4 years old, indicating that iron deficiency was not a factor in the study. At 35.2 μg/dl blood lead, 50 percent of the children had significantly elevated FEP levels (2 standard deviations above the reference mean FEP).

At blood lead levels below 30-40 μg/dl, any assessment of the EP-blood lead relationship is strongly influenced by the relative analytical proficiency of measurements of both blood lead and EP. The types of statistical analyses used are also important. In a recent detailed statistical study involving 2004 children, 1852 of whom had blood lead values below 30 μg/dl, segmental line and probit analysis techniques were employed to assess the dose-effect threshold and dose-response relationship. An average blood lead threshold for the effect using both statistical techniques was 16.5 μg/dl for the full group and for those subjects with blood lead below 30 μg/dl. The effect of iron deficiency was tested for and was removed. Of particular interest was the finding that blood lead values of 28.6 and 35.2 μg/dl corresponded to EP elevations of more than 1 or 2 standard deviations, respectively, above the reference mean in 50 percent of the children. Hence, fully half of the children had significant elevations of EP at blood lead levels around 30 μg/dl. From various reports, children and adult females appear to be more sensitive to lead's effects on EP accumulation at any given blood lead level; children are somewhat more sensitive than adult females.

Lead's effects on heme formation are not restricted to the erythropoietic system. Recent data indicate that the reduction of serum 1,25-dihydroxyvitamin D seen with even low-level lead exposure is apparently the result of lead-induced inhibition of the activity of renal

1-hydroxylase, a cytochrome P-450 mediated enzyme. Moreover lead inhibits renal ferrochelatase activity, which, with elevated kidney EP, leads to a reduction of heme available for heme-requiring enzymes such as renal 1-hydroxylase. Reduction in activity of the hepatic enzyme tryptophan pyrrolase and concomitant increases in plasma tryptophan as well as brain tryptophan, serotonin, and hydroxyindoleacetic acid have been shown to be associated with lead-induced reduction of the hepatic heme pool. The heme-containing hepatic protein cytochrome P-450 (an integral part of the hepatic mixed-function oxygenase system) is affected in humans and animals by lead exposure, especially acute intoxication. Reduced P-450 content correlates with impaired activity of detoxifying enzyme systems such as aniline hydroxylase and aminopyrine demethylase. It is also responsible for reduced 6β-hydroxylation of cortisol in children having moderate lead exposure.

Studies of organotypic chick and mouse dorsal root ganglion in culture show that the nervous system has heme biosynthetic capability and that not only is this capability reduced in the presence of lead but production of porphyrinic material is increased. In the neonatal rat, depending on the age at dosing and the duration of dosing, chronic lead exposure resulting in moderately elevated blood lead is associated with retarded increases in the hemoprotein cytochromes and with disturbed electron transport in the developing cerebral cortex. These data parallel effects of lead on ALA-D activity and ALA accumulation in neural tissue. When both of these effects are viewed in the toxicokinetic context of increased retention of lead in both developing animals and children, there is an obvious and serious potential for impaired heme-based metabolic function in the nervous system of lead-exposed children.

As can be concluded from the above discussion, the health significance of ZPP accumulation rests with the fact that it is evidence of impaired heme and hemoprotein formation in many tissues that arises from entry of lead into mitochondria. Elevation of EP in children at relatively low blood lead levels is considered by the pediatric medicine community to be a matter of concern, and the Centers for Disease Control in their recent statement on lead poisoning in children (U.S. Centers for Disease Control, 1985) have noted that a blood lead level above 25 μg/dl along with an EP level above 35 μg/dl whole blood is to be taken as early evidence of lead toxicity. Such evidence for reduced heme synthesis is consistent with a great deal of data documenting lead-associated effects on mitochondria. The relative value of the lead-ZPP relationship in erythropoietic tissue as an index of this effect in other tissues hinges on the relative sensitivity of the erythropoietic system compared with other organ systems. One study of rats exposed over their lifetime to low levels of lead demonstrated that protoporphyrin accumulation in renal tissue was already significant at levels of lead exposure which produced little change in erythrocyte porphyrin levels.

Other steps in the heme biosynthesis pathway are also known to be affected by lead, although these have not been as well studied on a biochemical or molecular level. Coproporphyrin levels are increased in urine, reflecting active lead intoxication. Lead also affects the activity of the enzyme uroporphyrinogen-I-synthetase in experimental animal systems, resulting in an accumulation of its substrate, porphobilinogen. The erythrocyte enzyme has been reported to be much more sensitive to lead than the hepatic species, presumably accounting for much of the accumulated substrate. Unlike the case with experimental animals, lead-exposed humans show no rise in urinary porphobilinogen, which is a differentiating characteristic of lead intoxication versus the hepatic porphyrias. Ferrochelatase is an intramitochondrial enzyme, and impairment of its activity either directly by lead or via impairment of iron transport to the enzyme is evidence of the presence of lead in mitochondria.

12.3.6.2 Lead Effects on Erythropoiesis and Erythrocyte Physiology. Anemia is a manifestation of chronic lead intoxication and is characterized as mildly hypochromic and usually normocytic. It is associated with reticulocytosis, owing to shortened cell survival, and the variable presence of basophilic stippling. Its occurrence is due to both decreased production and increased rate of destruction of erythrocytes. In young children (<4 yr old), iron deficiency anemia is exacerbated by lead uptake, and vice versa. Hemoglobin production is negatively correlated with blood lead in young children, in whom iron deficiency may be a confounding factor, as well as in lead workers. In one study, blood lead values that were usually below 80 µg/dl were inversely correlated with hemoglobin content. In these subjects no iron deficiency was found. The blood lead threshold for reduced hemoglobin content is about 50 µg/dl in adult lead workers and somewhat lower (about 40 µg/dl) in children.

The mechanism of lead-associated anemia appears to be a combination of reduced hemoglobin production and shortened erythrocyte survival due to direct cell injury. Lead's effects on hemoglobin production involve disturbances of both heme and globin biosynthesis. The hemolytic component to lead-induced anemia appears to be caused by increased cell fragility and increased osmotic resistance. In one study using rats, the hemolysis associated with vitamin E deficiency, via reduced cell deformability, was exacerbated by lead exposure. The molecular basis for increased cell destruction rests with inhibition of (Na^+, K^+)-ATPase and pyrimidine-5'-nucleotidase. Inhibition of the former enzyme leads to cell "shrinkage" and inhibition of the latter results in impaired pyrimidine nucleotide phosphorolysis and disturbance of the activity of the purine nucleotides necessary for cellular energetics.

12.3.6.3 Effects of lead on erythropoietic pyrimidine metabolism. In lead intoxication, the presence of both basophilic stippling and anemia with a hemolytic component is due to inhibition by lead of the activity of pyrimidine-5'-nucleotidase (Py-5-N), an enzyme that mediates the dephosphorylation of pyrimidine nucleotides in the maturing erythrocyte. Inhibition of this enzyme by lead has been documented in lead workers, lead-exposed children, and experimental animal models. In one study of lead-exposed children, there was a negative correlation between blood lead and enzyme activity, with no clear response threshold. A related report noted that, in addition, there was a positive correlation between cytidine phosphate and blood lead and an inverse correlation between pyrimidine nucleotide and enzyme activity.

The metabolic significance of Py-5-N inhibition and cell nucleotide accumulation is that they affect erythrocyte stability and survival as well as potentially affect mRNA and protein synthesis related to globin chain synthesis. Based on one study of children, the threshold for the inhibition of Py-5-N activity appears to be about 10 µg/dl blood lead. Lead's inhibition of Py-5-N activity and a threshold for such inhibition are not by themselves the issue. Rather, the issue is the relationship of such inhibition to a significant level of impaired pyrimidine nucleotide metabolism and the consequences for erythrocyte stability and function. The relationship of Py-5-N activity inhibition by lead to accumulation of its pyrimidine nucleotide substrate is analogous to lead's inhibition of ALA-D activity and accumulation of ALA.

12.3.6.4 Effects of Alkyl Lead Compounds on Heme Biosynthesis and Erythropoiesis. Tetraethyl lead and tetramethyl lead, components of leaded gasoline, undergo transformation *in vivo* to neurotoxic trialkyl metabolites as well as further conversion to inorganic lead. Hence, one might anticipate that exposure to such agents may result in effects commonly associated with inorganic lead, particularly in terms of heme synthesis and erythropoiesis. Various surveys and case reports show that the habit of sniffing leaded gasoline is associated with chronic lead intoxication in children from socially deprived backgrounds in rural or remote areas. Notable in these subjects is evidence of impaired heme biosynthesis, as indexed by significantly reduced ALA-D activity. In several case reports of frank lead toxicity from habitual leaded gasoline sniffing, effects such as basophilic stippling in erythrocytes and significantly reduced hemoglobin have also been noted.

12.3.6.5 Relationships of Lead Effects on Heme Synthesis to Neurotoxicity. The role of lead-associated disturbances of heme biosynthesis as a possible factor in neurological effects of lead is of considerable interest due to the following: (1) similarities between classical signs of lead neurotoxicity and several neurological components of the congenital disorder acute intermittent porphyria; and (2) some of the unusual aspects of lead neurotoxicity. There are three possible points of connection between lead's effects on heme biosynthesis and the nervous system. Associated with both lead neurotoxicity and acute intermittent porphyria is the common feature of excessive systemic accumulation and excretion of ALA. In addition, lead neurotoxicity reflects, to some degree, impaired synthesis of heme and hemoproteins involved in crucial cellular functions; such an effect on heme is now known to be relevant within neural tissue as well as in non-neural tissue.

Available information indicates that ALA levels are elevated in the brains of lead-exposed animals and arise through *in situ* inhibition of brain ALA-D activity or through transport of ALA to the brain after formation in other tissues. ALA is known to traverse the blood-brain barrier. Hence, ALA is accessible to, or formed within, the brain during lead exposure and may express its neurotoxic potential.

Based on various *in vitro* and *in vivo* neurochemical studies of lead neurotoxicity, it appears that ALA can inhibit release of the neurotransmitter gamma-aminobutyric acid (GABA) from presynaptic receptors at which ALA appears to be very potent even at low levels. In an *in vitro* study, agonist behavior by ALA was demonstrated at levels as low as 1.0 μM ALA. This *in vitro* observation supports results of a study using lead-exposed rats in which there was inhibition of both resting and K^+-stimulated release of preloaded ^{3}H-GABA from nerve terminals. The observation that *in vivo* effects of lead on neurotransmitter function cannot be duplicated with *in vitro* preparations containing added lead is further evidence of an effect of some agent (other than lead) that acts directly on this function. Human data on lead-induced associations between disturbed heme synthesis and neurotoxicity, while limited, also suggest that ALA may function as a neurotoxicant.

A number of studies strongly suggest that lead-impaired heme production itself may be a factor in the toxicant's neurotoxicity. In porphyric rats treated also with phenobarbital, both lead and the organic agent DDEP inhibit tryptophan pyrrolase activity owing to reductions in the hepatic heme pool, thereby leading to elevated levels of tryptophan and serotonin in the brain. Such elevations are known to induce many of the neurotoxic effects also seen with lead exposure. Of great interest is the fact that heme infusion in these animals reduces brain levels of these substances and also restores enzyme activity and the hepatic heme pool. It remains to be demonstrated that use of lead alone, without enzyme induction, would show similar effects. Another line of evidence for the heme-basis of lead neurotoxicity is that mouse dorsal root ganglion in culture manifests morphological evidence of neural injury with rather low lead exposure, but such changes are largely prevented with co-administration of heme. Finally, studies also show that heme-requiring cytochrome C production is impaired along with operation of the cytochrome C respiratory chain in the brain when neonate rats are exposed to lead.

12.3.6.6 Summary of Effects of Lead on Vitamin D Metabolism There has recently been a growing awareness of the interactions of lead and the vitamin D-endocrine system. A recent study has found that children with blood lead levels of 33-120 μg/dl showed significant reductions in serum levels of the hormonal metabolite 1,25-dihydroxyvitamin D ($1,25-(OH)_2D$). This inverse dose-response relationship was found throughout the range of measured blood lead values, 12-120 μg/dl, and appeared to be the result of lead's effect on the production of the vitamin D hormone. The 1,25-$(OH)_2D$ levels of children with blood lead levels of 33-55 μg/dl corresponded to the levels that have been observed in children with severe renal dysfunction. At higher blood lead levels (>62 μg/dl), the 1,25-$(OH)_2D$ values were similar to those that have been measured in children with various inborn metabolic disorders. Chelation therapy of the lead-poisoned children (blood lead levels >62 μg/dl) resulted in a return to normal 1,25-$(OH)_2D$ levels within a short period.

In addition to its well-known actions on bone remodeling and intestinal absorption of minerals, the vitamin D hormone has several other physiological actions at the cellular level. These include cellular calcium homeostasis in virtually all mammalian cells and associated calcium-mediated processes that are essential for cellular integrity and function. In addition, the vitamin D hormone has newly recognized functions that involve cell differentiation, immunoregulatory capacity, and other roles distinct from mineral metabolism. It is reasonable to conclude, therefore, that impaired production of 1,25-$(OH)_2D$ can have profound and pervasive effects on tissues and cells of diverse type and function throughout the body.

12.4 NEUROTOXIC EFFECTS OF LEAD

12.4.1 Introduction

Historically, neurotoxic effects have long been recognized as being among the more severe consequences of human lead exposure (Tanquerel des Planches, 1839; Stewart, 1895; Prendergast, 1910; Oliver, 1911; Blackfan, 1917). Since the early 1900s, extensive research has focused on the elucidation of lead exposure levels associated with the induction of various types of neurotoxic effects and related issues, such as critical exposure periods for their induction and their persistence or reversibility. Such research, spanning more than 50 years, has provided increasing evidence indicating that progressively lower lead exposure levels, previously accepted as "safe," are actually sufficient to cause notable neurotoxic effects.

The neurotoxic effects of extremely high exposures, resulting in blood lead levels in excess of 80-100 μg/dl, have been well documented, especially in regard to increased risk for fulminant lead encephalopathy (a well-known clinical syndrome characterized by overt symptoms such as gross ataxia, persistent vomiting, lethargy, stupor, convulsions, and coma). The persistence of neurological sequelae in cases of non-fatal lead encephalopathy has also been well established. The neurotoxic effects of subencephalopathic lead exposures in both human adults and children, however, continue to represent a major area of interest and controversy. Reflecting this, much research during the past 10-15 years has focused on the delineation of exposure-effect relationships for the following: (1) the occurrence of overt signs and symptoms of neurotoxicity in relation to other indicators of subencephalopathic overt lead intoxication; and (2) the manifestation of more subtle, often difficult-to-detect indications of altered neurological functions in apparently asymptomatic (i.e., not overtly lead-poisoned) individuals.

The present assessment critically reviews the available scientific literature on the neurotoxic effects of lead, first evaluating the results of human studies bearing on the subject and then examining pertinent animal toxicology studies. The discussion of human studies is divided into two major subsections focusing on neurotoxic effects of lead exposure in (1) adults and (2) children. Lead's effects on both the central nervous system (CNS) and the peripheral nervous system (PNS) are discussed in each case. In general, only relatively brief overview summaries are provided in regard to findings bearing on the effects of extremely high-level exposures resulting in encephalopathy or other frank signs or symptoms of overt lead intoxication. Studies concerning the effects of lower-level lead exposures are assessed in more detail, especially those dealing with non-overtly lead intoxicated children. As for the animal toxicology studies, particular emphasis is placed on the review of studies that help to address certain important issues raised by the human research findings, rather than attempting an exhaustive review of all animal toxicology studies concerning the neurotoxic effects of lead.

12.4.2 Human Studies

Defining exposure-effect or dose-response relationships between lead and particular neurotoxic responses in humans involves two basic steps. First, there must be an assessment of the internal lead burden resulting from external doses of lead received via various routes of exposure (such as air, water, food, occupational hazards, house dust, etc.). Internal lead burdens may be indexed by lead concentrations in blood, teeth, or other tissue, or by other biological indicators. The second step involves an assessment of the relationship of internal exposure indices to behavioral or other types of neurophysiological responses. The difficulty of this task is reflected by current controversies over existing data. Studies vary greatly in the quality of design, precision of assessment instruments, care in data collection, and appropriateness of statistical analyses employed. Many of these methodological problems are broadly common to research on toxic agents in general and not just to lead alone.

Although epidemiological studies of lead's effects have immediate environmental relevance at the human level, difficult problems are often associated with the interpretation of the findings, as noted in several reviews (Bornschein et al., 1980; Cowan and Leviton, 1980; Rutter, 1980; Valciukas and Lilis, 1980; Needleman and Landrigan, 1981). The main problems are the following: (1) inadequate markers of exposure to lead; (2) insensitive measures of performance; (3) bias in selection of subjects; (4) inadequate handling of confounding covariates; (5) inappropriate statistical analyses; (6) inappropriate generalization and interpretation of results; and (7) the need for "blind" evaluations by experimenters and technicians. Each of these problems is briefly discussed below.

Each major exposure route--food, water, air, dust, and soil--contributes to a person's total daily intake of lead (see Chapters 7 and 11). The relative contribution of each exposure route, however, is difficult to ascertain; neurotoxic endpoint measurements, therefore, are most typically evaluated in relation to one or another indicator of overall internal lead body burden. Subjects in epidemiological studies may be misclassified as to exposure level unless careful choices of exposure indices are made based upon the hypotheses to be tested, the accuracy and precision of the biological media assays, and the collection and assay procedures employed. Chapter 9 of this document evaluates different measures of internal exposure to lead and their respective advantages and disadvantages. The most commonly used measure of internal dose is blood lead concentration, which varies as a function of age, sex, race, geographic location, and exposure. The blood lead level is a useful marker of current exposure but generally does not reflect cumulative body lead burdens as well as lead levels in teeth. Hair lead levels, measured in some human studies, are not viewed as reliable indicators of internal body burdens at this time. Future research may identify a more standard exposure index, but it appears that a risk classification similar to that of the U.S. Centers for Disease Control (1978) in terms of blood lead and free erythrocyte protoporphyrin (FEP) levels will continue in the foreseeable future to be the standard approach most often used for lead exposure screening and evaluation. Much of the discussion below is, therefore, focused on defining dose-effect relationships for human neurotoxic effects in terms of blood lead levels; some ancillary information on pertinent tooth lead levels is also discussed.

The frequency and timing of sampling for internal lead burdens represent another important factor in evaluating studies of lead effects on neurological and behavioral functions. For example, epidemiological studies often rely on blood lead and/or erythrocyte protoporphyrin (EP) levels determined at a single point in time to retrospectively estimate or characterize internal exposure histories of study populations that may have been exposed in the past to higher levels of lead than those indicated by a single current blood sample. Relatively few prospective studies exist that provide highly reliable estimates of critical lead exposure levels associated with observed neurotoxic effects in human adults or children, especially in regard to the effects of subencephalopathic lead exposures. Some prospective longitudinal studies on the effects of lead on early development of infants and young children are currently in progress, but results of these studies are only beginning to become available (see Section 12.4.2.2.2.5 below). The present assessment of the neurotoxic effects of lead in humans must, therefore, rely most heavily on published epidemiological studies which typically provide exposure history information of only limited value in defining exposure-effect relationships and less-than-optimum cross-sectional study designs.

Key variables that have emerged in determining effects of lead on the nervous system include (1) duration and intensity of exposure and (2) age at exposure. Much evidence suggests that young organisms with developing nervous systems are more vulnerable than adults with fully matured nervous systems. Particular attention is, therefore, accorded below to discussion of neurotoxic effects of lead in children as a special group at risk.

Precision of measurement is a critical methodological issue, especially when research on neurotoxicity leaves the laboratory setting. Neurotoxicity is often measured indirectly with psychometric or neurometric techniques in epidemiological studies (Valciukas and Lilis, 1980). The accuracy with which these tests reflect what they purport to measure (validity) and the degree to which they are reproducible (reliability) are issues central to the science of measurement theory. Many cross-sectional population studies make use of instruments that are only brief samples of behavior thought to be representative of some relatively constant underlying traits, such as intelligence. Standardization of tests is the subject of much research in psychometrics. The quality and precision of specific test batteries have been particularly controversial issues in evaluating possible effect levels for neurotoxic effects of lead exposure in children. Table 12A (Appendix 12A) lists some of the major tests used, together with their advantages and weaknesses. The following review places most weight on results obtained with age-normed, standardized psychometric test instruments, and well-controlled, standardized nerve conduction velocity tests. Other measures, such as reaction time, finger tapping, and certain electrophysiological measures (e.g., cortical evoked and slow-wave potentials) are potentially more sensitive indices, but are still experimental measures whose clinical utility and psychometric properties with respect to the neurobehavioral toxicity of lead remain to be more fully explored.

Selection bias is a critical issue in epidemiological studies in which attempts are made to generalize from a small sample to a large population. Volunteering to participate in a study and attendance at special clinics or schools are common forms of selection bias that often limit how far the results of such studies can be generalized. These factors may need to be balanced in lead neurotoxicity research since reference groups are often difficult to find because of the pervasiveness of lead in the environment and the many non-lead covariates that also affect performance. Selection bias and the effects of confounding can be reduced by choosing a more homogeneous stratified sample, but the generalizability of the results of such cohort studies is thereby limited.

Perhaps the greatest methodological concern in epidemiological studies is controlling for confounding covariates, so that residual effects can be more confidently attributed to lead. Among adults, the most important covariates are age, sex, race, educational level, exposure history, alcohol intake, total food intake, dietary calcium and iron intake, and urban versus rural styles of living (Valciukas and Lilis, 1980). Among children, a number of developmental covariates are additionally important: parental socioeconomic status (Needleman et al., 1979); maternal IQ (Perino and Ernhart, 1974); pica (Barltrop, 1966); quality of the care-giving environment (Hunt et al., 1982; Milar et al., 1980); dietary iron and calcium intake; vitamin D levels; body fat and nutrition (Mahaffey and Michaelson, 1980; Mahaffey, 1981); and age at exposure. Preschool children below the age of 3-5 years appear to be particularly vulnerable, in that the rate of accumulation of even a low body lead burden is higher for them than for adults (National Academy of Sciences, Committee on Lead in the Human Environment, 1980). Potential confounding effects of covariates become particularly important when trying to interpret threshold effects of lead exposure. Each covariate alone may not be significant, but, when combined, may interact to pose a cumulative risk which could result in under- or overestimation of a small effect of lead.

Statistical considerations important not only to lead but to all epidemiological studies include adequate sample size (Hill, 1966), the use of multiple regression (Cohen and Cohen, 1975), and the use of multivariate analyses (Cooley and Lohnes, 1971). Regarding sample size, false negative conclusions are at times drawn from small studies with low statistical power. It is often difficult and expensive to use large sample sizes in complex research such as that on lead neurotoxicity. This fact makes it all the more important to use sensitive assessment instruments which have a high level of discriminating power and can be combined into factors for multivariate analysis. Multiple statistical comparisons can then be made while reducing the likelihood of finding a certain number of significant differences by chance alone. This is a serious problem, because near-threshold effects are often small and variable.

A final crucial issue in this and other research revolves around the care taken to assure that investigators are isolated from information that might identify subjects in terms of their lead exposure levels at the time of assessment and data recording. Unconscious biases, nonrandom errors, and arbitrary data correction and exclusion can be ruled out only if a study is performed under blind conditions or, preferably, double-blind conditions.

With the above methodological considerations in mind, the following sections evaluate pertinent human studies. The discussion includes an overview of lead exposure effects in adults, followed by a more detailed assessment of neurotoxic effects of lead exposures in children.

12.4.2.1 Neurotoxic Effects of Lead Exposure in Adults.

12.4.2.1.1 Overt lead intoxication in adults. Severe neurotoxic effects of extreme exposures to high levels of lead, especially for prolonged periods that produce overt signs of acute lead intoxication, are well documented in regard to both adults and children. The most profound (CNS) effects in adults have been referred to for many years as the clinical syndrome of lead encephalopathy, described in detail by Aub et al. (1926), Cantarow and Trumper (1944), Cumings (1959), and Teisinger and Stýblová (1961). Early features of the syndrome that may develop within weeks of initial exposure include dullness, restlessness, irritability, poor attention span, headaches, muscular tremor, hallucinations, and loss of memory. These symptoms may progress to delirium, mania, convulsions, paralysis, coma, and death. The onset of such symptoms can often be quite abrupt, with convulsions, coma, and even death occurring very rapidly in patients who shortly before appeared to exhibit much less severe or no symptoms of acute lead intoxication (Cumings, 1959; Smith et al., 1938). Symptoms of lead encephalopathy indicative of severe CNS damage and posing a threat to life are generally not seen in adults except at blood lead levels well in excess of 120 μg/dl (Kehoe, 1961a,b,c). Other data (Smith et al., 1938) suggest that acute lead intoxication, including severe gastrointestinal symptoms and/or signs of encephalopathy can occur in some adults at blood lead levels around 100 μg/dl, but ambiguities make the data difficult to interpret.

In addition to the above CNS effects, lead also clearly damages peripheral nerves at toxic, high-exposure levels that predominantly affect large myelinated nerve fibers (Vasilescu, 1973; Feldman et al., 1977; Englert, 1980). Pathologic changes in peripheral nerves, as shown in animal studies, can include both segmental demyelination and, in some fibers, axonal degeneration (Fullerton, 1966). The former types of changes appear to reflect lead's effects on

Schwann cells, with concomitant endoneurial edema and disruption of myelin membranes (Windebank and Dyck, 1981). Apparently, lead induces a breakdown in the blood-nerve barrier which allows lead-rich edema fluid to enter the endoneurium (Dyck et al., 1980; Windebank et al., 1980). Remyelination observed in animal studies suggests either that such lead effects may be reversible or that not all Schwann cells are affected equally (Lampert and Schochet, 1968; Ohnishi and Dyck, 1981). Reports of plantar arch deformities due to old peripheral neuropathies (Emmerson, 1968), however, suggest that lead-induced neuropathies of sufficient severity in human adults could result in permanent peripheral nerve damage. Morphologically, peripheral neuropathies are usually detectable only after prolonged high exposure to lead, with distinctly different sensitivities and histological differences existing among mammalian species. In regard to man, as an example, Buchthal and Behse (1979, 1981), using nerve biopsies from a worker with frank lead neuropathy (blood lead = 150 µg/dl), found histological changes indicative of axonal degeneration in association with reductions in nerve conduction velocities that corresponded to loss of large fibers and decreased amplitude of sensory potentials.

Data from numerous studies provide a basis by which to estimate lead exposure levels at which adults exhibit overt signs or symptoms of neurotoxicity and to compare such levels with those associated with other types of signs and symptoms indicative of overt lead intoxication (Sakurai et al., 1974; Lilis et al., 1977; Tola and Nordman, 1977; Irwig et al., 1978a,b; Dahlgren, 1978; Baker et al., 1979; Haenninen et al., 1979; Spivey et al., 1979; Fischbein et al., 1980; Hammond et al., 1980; Kirkby et al., 1983). These studies evaluated rates of various clinical signs and symptoms of lead intoxication across a wide range of lead exposures among occupationally exposed smelter and battery plant workers.

Considerable individual biological variability is apparent among various study populations and individual workers in terms of observed lead levels associated with overt signs and symptoms of lead intoxication, based on comparisons of exposure-effect and dose-response data from the available studies. For example, Irwig et al. (1978a,b) and Zielhuis and Wibowo (1976) discuss data for black South African lead workers indicative of increased prevalence of neurological symptoms at 110 µg/dl and gastrointestinal symptoms at blood lead levels in excess of 60 µg/dl. Analogously, Hammond et al. (1980) reported significant increases in neurological (both CNS and PNS) and gastrointestinal symptoms among American smelter workers with blood lead levels often exceeding 80 µg/dl, but not among workers whose exposure histories did not include levels above 80 µg/dl. Also, Kirkby et al. (1983) found no significant differences between 96 long-term lead smelter workers and 96 matched control subjects in prevalence of self-reported symptoms of fatigue, headache, nervousness, sleep disturbance, constipation, or colic. Blood lead levels for the lead workers averaged 51 µg/dl (range 13-91 µg/dl), whereas the control group averaged 11 µg/dl (range 6-16 µg/dl).

In contrast to the above results, many other investigators have reported neurologic symptoms and other overt signs and symptoms of lead toxicity at blood lead levels ranging well below 80 μg/dl. Lilis et al. (1977), for instance, found that CNS symptoms (tiredness, sleeplessness, irritability, headaches) were reported by 55 percent and muscle or joint pain by 39 percent of a group of lead smelter workers whose blood lead levels had never been observed to exceed 80 μg/dl. Low hemoglobin levels (<14 g/dl) were found in more than 33 percent of these workers. In addition, Spivey et al. (1979) reported significantly increased neurological (mainly CNS, but some PNS) symptoms and joint pain among a group of 69 lead workers with mean ± standard deviation blood lead levels of 61.3 ± 12.8 μg/dl in comparison to a control group with 22.0 ± 5.9 μg/dl blood lead values. Haenninen et al. (1979) similarly reported significantly increased neurological (both CNS and PNS) and gastrointestinal symptoms among 25 lead workers with maximum observed blood lead levels of 50-69 μg/dl and significantly increased CNS symptoms among 20 lower exposure workers with maximum blood lead values below 50 μg/dl. Both groups were compared against a referent control group (N = 23) with blood lead values of 11.9 ± 4.3 μg/dl.

Additional studies (Baker et al., 1979; Fischbein et al., 1980; Zimmermann-Tansella et al., 1983) provide evidence of overt signs or symptoms of neurotoxicity occurring at lead exposure levels still lower than those indicated above. Baker et al. (1979) studied dose-response relationships between clinical signs and symptoms of lead intoxication among lead workers in two smelters. No overt toxicity was observed at blood lead levels below 40 μg/dl. However, 13 percent of those workers with blood lead values in the range 40-79 μg/dl had extensor muscle weakness or gastrointestinal symptoms; and anemia occurred in 5 percent of the workers with 40-59 μg/dl blood lead levels, in 14 percent with levels of 60-79 μg/dl, and in 36 percent with blood lead levels exceeding 80 μg/dl. Also, Fischbein et al. (1980), in a study of 90 cable splicers intermittently exposed to lead, found higher zinc protoporphyrin levels (an indicator of impaired heme synthesis associated with lead exposure) among workers reporting CNS or gastrointestinal symptoms than among other cable splicers not reporting such symptoms. Only 5 percent of these workers had blood lead levels in excess of 40 μg/dl, and the mean ± standard deviation blood lead levels for the 26 reporting CNS symptoms were 28.4 ± 7.6 μg/dl and 30 ± 9.4 μg/dl for the 19 reporting gastrointestinal symptoms. However, caution must be exercised in accepting these latter blood levels as being representative of average or maximum lead exposures of this worker population, in view of the highly intermittent nature of their exposure and the likelihood of much higher peaks in their blood lead levels than those coincidentally measured at the time of their blood sampling.

Lastly, Zimmermann-Tansella et al. (1983) have independently confirmed and extended previously described findings of Haenninen et al. (1979). Three groups of 20 men each were matched on age, education, marital status, chronic illnesses, personality characteristics, and length of employment. The control group had no history of occupational exposure to lead (mean blood lead level = 20.4 ± 6 μg/dl). The lead-exposed groups were composed of workers from an electric storage battery plant, with the low-lead group averaging 31.7 ± 2.9 μg/dl (range: 26-35 μg/dl) and the high-lead group 52.5 ± 5.1 μg/dl (range: 45-60 μg/dl). None ever exceeded 60 μg/dl. In conjunction with other psychological testing (Campara et al., 1984), two questionnaires were given that asked about a variety of emotional, neurological, and gastrointestinal symptoms similar to symptoms covered by the questionnaire used by Haenninen et al. (1979). The most clear-cut effects, in terms of significant and consistent dose-response trends, were found in physical symptoms (such as loss of appetite, paresthesis in lower limbs, weakness of upper limbs, and dropping of objects) with the most marked increases seen in rates of neurological symptoms in the high-lead group. Coupled with the increased symptom rates observed by Zimmermann-Tansella et al. (1983) were observations reported by Campara et al. (1984) indicating that the high-lead workers did significantly more poorly on a variety of psychometric tests (e.g., the WAIS), with general performance (on cognitive and visual-motor coordination tasks) and verbal reasoning ability most markedly impaired. These findings, consistent with earlier results of Haenninen et al. (1978, 1979), indicate that overt neurological symptoms and impaired CNS functioning, as well as gastrointestinal symptoms, occur in adults at blood lead levels of 45-60 μg/dl.

Overall, the results reviewed above appear to support the following conclusions: (1) overt signs and symptoms of neurotoxicity in adults are manifested at roughly comparable lead exposure levels as other types of overt signs and symptoms of lead intoxication, such as gastrointestinal complaints; (2) neurological signs and symptoms are indicative of both central and peripheral nervous system effects; (3) such overt signs and symptoms, both neurological and otherwise, occur at markedly lower blood lead levels than levels previously thought to be "safe" for adults; and (4) lowest observed effect levels for the neurological signs and symptoms in adults can most credibly be stated to be in the 40-60 μg/dl range. Insufficient information currently exists to estimate with confidence to what extent or for how long such overt signs and symptoms persist in adults after termination of precipitating external lead exposures, but at least one study (Dahlgren, 1978) has reported abdominal pain persisting as long as 29 months after exposure termination among 15 smelter workers, including four whose blood lead levels were between 40 and 60 μg/dl while working.

12.4.2.1.2 Non-overt lead intoxication in adults. Of special importance for establishing standards for exposure to lead is the question of whether exposures lower than those producing overt signs or symptoms of lead intoxication result in less obvious neurotoxic effects in otherwise apparently healthy individuals. Attention has focused in particular on whether exposures leading to blood lead levels below 80-100 μg/dl may lead to behavioral deficits or other neurotoxic effects in the absence of classical signs of overt lead intoxication.

In adults, one might expect neurobehavioral deficits to be reflected by performance measures in the workplace, such as higher rates of absences or reduced psychomotor performance among occupationally exposed lead workers. Some epidemiological studies have investigated possible relationships between elevated blood lead and general health as indexed by records of sick absences certified by physicians (Araki et al., 1982; Robinson, 1976; Shannon et al., 1976; Tola and Nordman, 1977). However, sickness absence rates are generally poor epidemiologic outcome measures that may be confounded by many variables and are difficult to relate specifically to lead exposure levels. Much more useful are studies that evaluate direct measurements of central or peripheral neurological functions in relation to lead exposure.

A number of studies have employed sensitive neurological and/or psychometric testing procedures in an effort to demonstrate specific lead-induced neurobehavioral effects in adults. Disturbances in oculomotor function have been found in two studies of lead-exposed workers. The first, a prospective investigation by Baloh et al. (1979), found significantly decreased saccade accuracy and similar but nonsignificant differences in saccade velocity and delay times in lead workers (mean blood lead: ~61 μg/dl) compared to controls. A follow-up examination (Spivey et al., 1980) essentially replicated the original findings. A more recent investigation of saccadic eye movements by Glickman et al. (1984) also found highly significant decreases in saccade accuracy and increases in overshoots among lead workers (mean blood lead: 57 μg/dl), particularly younger workers. The difference in saccade velocity fell just short of statistical significance overall ($p = 0.056$), but was highly significant ($p < 0.004$) in the 20-29 year age group. Also, velocity and ZPP were significantly correlated overall ($r = -0.40$, $p < 0.005$).

Morgan and Repko (1974) reported deficits in hand-eye coordination and reaction time in an extensive study of behavioral functions in 190 lead-exposed workers (mean blood lead level = 60.5 ± 17.0 μg/dl). The majority of the subjects had been exposed between 5 and 20 years. In a similar study, however, Milburn et al. (1976) found no differences between control and lead-exposed workers on numerous psychometric and other performance tests. On the other hand, several other studies (Arnvig et al., 1980; Grandjean et al., 1978; Haenninen et al., 1978; Hogstedt et al., 1983; Mantere et al., 1982; Valciukas et al., 1978) have found disturbances in reaction time, visual motor performance, hand dexterity, IQ test/cognitive performance, mood, nervousness, or coping in lead workers with blood lead levels of 50-80 μg/dl. Hogstedt et al. (1983) also found impaired memory and learning ability in workers with time-weighted average blood lead levels of 27-52 μg/dl. Furthermore, Baker et al. (1983) found significantly increased rates of depression, confusion, anger, fatigue, and tension among workers with blood lead levels above 40 μg/dl, who did not differ from referent control workers in terms of reported incidence of abdominal colic or other gastrointestinal symptoms characteristic of overt lead intoxication. Other aspects of neurobehavioral function in the same workers were also found to be impaired, including verbal concept formation, memory, and visual/motor performance. A graded dose-effect relationship for non-overt CNS lead effects in otherwise asymptomatic adults is indicated by such studies.

In addition to the above studies indicative of psychoneural dysfunctions in non-overtly lead intoxicated adults, numerous investigations have examined peripheral nerve function by measuring the conduction velocity of electrically stimulated nerves in the arm or leg. Nerve conduction velocity (NCV) provides a readily accessible indication of neurophysiological function in sensory as well as motor nerves. However, nerve temperature (and to some extent, skin temperature and room temperature), age, and limb length affect conduction velocity and thus may confound NCV measurements. Table 12-1 summarizes several studies of groups of lead-exposed subjects and notes what was done to deal with the above confounding factors.

Of particular note are the positive findings of decreased NCVs at blood lead levels of 30-50 µg/dl from a prospective occupational study by Seppäläinen et al. (1983) in contrast to the negative findings at blood lead levels of 60-80 µg/dl from a prospective study by Spivey et al. (1980). Also contrasting are the results of two cross-sectional studies: Rosén et al. (1983) observed significant slowing of NCVs as a function of average blood lead levels monitored over 9 years, whereas Triebig et al. (1984) reported no apparent dose-effect relationship on NCVs except at blood lead levels exceeding 70 µg/dl. Although Triebig et al. (1984) did not examine as many neurophysiological variables as Rosén et al. (1983), they did incorporate many more lead-exposed subjects (N = 133) compared to most occupational lead studies of NCV. Triebig et al. (1984) also noted that the earlier findings of Seppäläinen et al. (1975, 1979) were confounded by age effects, since their lead-exposed subjects were older than the controls, but no correction was made for the normal slowing of NCV with increasing age. However, even if one allows a decline of approximately 2 m/sec in NCV for each 10 years' increase in age (based on Triebig et al., 1984), the age differences in Seppäläinen et al. (1983) would not appear to be sufficient to account for the significant declines in NCV that they found, except possibly at the four-year stage of their longitudinal study. Moreover, Rosén et al. (1983) did include age as a covariate in their analyses and still found significant effects of lead on NCV and other neurophysiological variables.

One difficulty in drawing conclusions from the studies presented in Table 12-1 is the lack of consistency among studies, either in the nerves examined or in the significance of results obtained. No one nerve has been consistently used in all the studies dealing with lead exposure and NCV measurements. Even when a particular nerve is singled out for consideration, the results may not be in complete agreement. For example, Seppäläinen et al. (1975, 1979) initially found the ulnar slow fiber NCVs to be a sensitive indicator of lead-induced impairment. But more recent work by Seppäläinen et al. (1983) and some other investigators (e.g.,

TABLE 12-1. SUMMARY OF STUDIES ON NERVE CONDUCTION VELOCITY IN GROUPS OF LEAD-EXPOSED SUBJECTS

Reference	Mean blood lead, μg/dl		Exposure period, yr. (range/±S.D.)	No. of subjects		Nerve*	NCV, m/sec		% Diff.	p	Comments
	Exp. (range/±S.D.)	Con.		Exp.	Con.		Exp.	Con.			
Seppäläinen et al. (1983)	16 (7-30)	10 (1-21)	0	23	23	Med - m	58.8	60.7	-3	N.S.	Prospective study followed workers from onset of employment (0-yr expos.) to 4 yr. Comparison values for years 1-4 are for "high" and "low" Exp. Ss with PbBs above or below median PbB (29-30 μg/dl). Ulnar-sf NCV was measured but not reported. Con. group 1.5 yr older than Exp. group at 0 and 1 yr; 0.7 yr younger at 2 yr; 4 yr younger at 4 yr.
						Med - s	63.7	64.2	-1	N.S.	
						Uln - m	60.7	59.3	+2	N.S.	
						Uln - s	62.1	62.7	-1	N.S.	
	31 (13-48)	10 (7-18)	1	23	23	Med - m	55.7	61.5	-9	<0.01	
						Med - s	60.5	64.2	-6	<0.05	
						Uln - m	57.0	61.5	-7	<0.01	
						Uln - s	59.2	64.6	-8	<0.01	
	30 (13-48)	10 (5-21)	2	15	15	Med - m	54.8	60.4	-9	<0.02	
						Med - s	58.5	60.7	-4	N.S.	
						Uln - m	59.3	62.2	-5	N.S.	
						Uln - s	59.9	60.8	-2	N.S.	
	27 (17-37)	7 (4-12)	4	10	10	Med - m	59.1	64.5	-8	<0.05	
						Med - s	59.9	61.1	-2	N.S.	
						Uln - m	62.8	62.9	0	N.S.	
						Uln - s	63.2	66.1	-4	N.S.	
Corsi et al. (1984)	28 (max: 71-180 4 yr or more earlier)	20	7 (0.08-37) (3-27 yr since last exposure)	38	23	Uln - m	54.5	57.9	-6	<0.05	Exp. group 2 yr older than Con. Skin temperature regulated. Multiple t-tests.
						Uln - sf	49.9	54.0	-8	<0.005	
						Per - m	48.7	51.3	-5	<0.01	
						Per - sf	45.2	48.5	-7	<0.005	
Johnson et al. (1980)	♀ 30(±14)	10(±4)	2.4(±1.5)	45	31	Uln - m	58.8	61.5	-4	N.S.	NCVs corrected for skin temperature and age.
						Per - m	52.3	55.2	-5	0.05	
	♂ 56(±13)	15(±6)	12.4(±10)	164	108	Uln - m	56.5	57.1	-1	N.S.	
						Per - m	49.4	50.4	-2	N.S.	
Seppäläinen et al. (1979)	34 actual 45 TWA† (max: <70)	10	~8	61	34	Med - m	59.6	61.0	-2	N.S.	Exp. group ~4 yr older than Con. Skin temperature usually <0.5°C higher in Exp. Ss. Ulnar-sf sig. slower even for Ss whose max. PbBs were <50 μg/dl.
						Med - s	62.0	65.3	-5	<0.005	
						Uln - m	60.7	60.5	0	N.S.	
						Uln - s	60.9	63.1	-4	N.S.	
						Uln - sf	42.5	45.9	-7	<0.005	
						Per - m	54.6	54.3	+1	N.S.	
						Tib - m	48.6	50.7	-4	<0.05	
						Sur - s	43.9	43.9	0	N.S.	

TABLE 12-1. (continued)

Reference	Mean blood lead, µg/dl Exp. (range/±S.D.)	Con.	Exposure period, yr. (range/±S.D.)	No. of subjects Exp.	Con.	Nerve*	NCV, m/sec Exp.	Con.	% Diff.	p	Comments
Seppäläinen et al. (1975)	40 (28-65)	10-13 (estimated)	4.6 (1-17)	26	26	Med - m	54.5	58.5	-7	<0.005	Con. group values obtained from separate studies.
				25	8	Med - s	59.5	56.3	+6	N.S.	
				25	26	Uln - m	55.0	58.1	-5	<0.01	
				26	22	Uln - sf	42.0	47.1	-11	<0.001	
				25	23	Uln - s	58.2	60.0	-3	N.S.	
				25	26	Per - m	50.6	52.0	-3	N.S.	
				26	19	Tib - m	43.4	44.6	-3	N.S.	
			Overall =	26	= 26						
Verberk (1976)	40	20	49 days	10	9	Uln - m	57.4	59.2	-3	N.S.	Volunteers ingested Pb. No info. on skin temperature or age comparisons. Residual error > difference between groups; ulnar-sf NCVs 12-13% faster in both groups compared to pre-exposure values.
						Uln - sf	46.2	50.8	-9	N.S.	
Rosén et al. (1983)	>40 (max: >55)	<25 (max: 30)	(0.5-28)	15 (intermediate PbB group N = 8)	16	Uln - m	57.0	58.1	-2	N.S.	NCV values adjusted for age. Cumulative exposure showed no apparent effect.
						Med - m	55.8	56.9	-2	N.S.	
						Fib - m	45.7	48.1	-5	0.019	
						Tib - m	44.0	45.7	-4	N.S.	
						Uln - sf	40.7	43.2	-6	N.S.	
						Med - s	48.7	48.7	0	N.S.	
						Sur - s	43.1	49.1	-12	0.022	
Bordo et al. (1982a,b)	42(±12) (avg. <50 during preceding 24 mo.)	16(±3)	4 (0.5-10)	62	27	Med - m	59.7	63.1	-5	<0.01	ANACOVA included age as covariate. Duration of expos. showed no effect.
						Med - s	59.8	63.5	-6	<0.01	
						Per - m	51.8	51.4	+1	N.S.	
Araki & Honma (1976)	45 (29-73)	12	18 (0.67-46)	19	39	Med - m	54.3	59.0	-8	<0.01	Room temperature measured, but not skin temperature. No info. on age of Con. Ss.
						Med - mx	64.1	67.1	-4	N.S.	
						Tib - m	44.7	50.0	-11	<0.01	

TABLE 12-1. (continued)

Reference	Mean blood lead, µg/dl		Exposure period, yr. (range/±S.D.)	No. of subjects		Nerve*	NCV, m/sec		% Diff.	p	Comments
	Exp. (range/±S.D.)	Con.		Exp.	Con.		Exp.	Con.			
Reichenbach (1979)	♀ 45(±13)	19(±2)	5 (0.25-11)	9	20	Uln - m	55.2	55.7	-1	N.S.	Ulnar NCV values corrected for skin temperature. Male groups age-matched, female Exp. group 3 yr older than Con. group. Although combined radial-sf values not reported as statistically significant, difference between female groups for radial-sf NCVs appears to be significant despite high variance.
				9	20	Uln - sf	48.5	49.4	-2	N.S.	
				9	18	Rad - m	62.1	65.2	-5	N.S.	
				7	16	Rad - sf	41.7	51.2	-19	N.S.	
			Overall =	9	20						
	♂ 70(±15)	22(±14)	13 (3-28)	22	20	Uln - m	52.4	53.6	-2	N.S.	
				22	20	Uln - sf	45.1	48.7	-7	<0.05	
				19	19	Rad - m	65.2	68.0	-4	N.S.	
				19	19	Rad - sf	51.3	49.7	+3	N.S.	
			Overall =	22	20						
Repko et al. (1978)	46 (<80 during preceding 5 yrs.)	18	9 (0.25-34)	80	43	Med - m	53.4	59.9	-11	0.00003	Skin temperature controlled and corrected for data analysis. Only sig. correlation between PbB and NCV was for ulnar-m.
				79	44	Uln - m	55.6	64.5	-14	0.00003	
				21	29	Uln - s	56.4	63.0	-10	0.015	
				36	29	Uln - sf	48.0	45.7	+5	N.S.	
				37	28	Tib - m	50.5	55.5	-9	0.0013	
Persson et al. (1979)	39 current (47 past average)	16-19 current (26 past average)	20.2 (±9.3)	58	58	Med - m	55.0	55.1	0	N.S.	Exp. group ~3 yr older than Con. group. No info. on skin temperature.
						Uln - m	55.2	56.1	-2	N.S.	
						Uln - sf	34.3	33.2	+3	N.S.	
Jeyaratnam et al. (1985)	49	16	?	46	64	Med - m	54.7	61.3	-11	<0.0001	Con. values obtained from separate independent study. Room temperature and skin impedance controlled, but not skin temp.
						Med - s	46.7	45.7	+2	N.S.	
						Sur - s	44.2	44.9	-2	N.S.	
						Tib - m	47.2	49.6	-5	<0.025	
Nielsen et al. (1982)	51 (13-91)	10 (6-16)	>9	89	21	Med - m	56	56	0	N.S.	Mean age of Exp. and Con. groups equal, but no info. on subsets of Ss used in different NCV tests.
				62	14	Rad - m	67	64	+5	N.S.	
				86	21	Per - m	48	48	0	N.S.	
			Overall =	95	= 21						
Araki et al. (1980)	52 (pre-treatment)	33 (post-treatment)	?	14	N/A	Med - m	52	58	-11	<0.01	Exp. Ss treated with EDTA to reduce PbB; no separate Con. group. Difference between Exp. and Con. values reflects change over 1-mo to 3-yr period after EDTA treatment.

TABLE 12-1. (continued)

Reference	Mean blood lead, µg/dl Exp. (range/±S.D.)	Mean blood lead, µg/dl Con. (range/±S.D.)	Exposure period, yr. (range/±S.D.)	No. of subjects Exp.	No. of subjects Con.	Nerve*	NCV, m/sec Exp.	NCV, m/sec Con.	% Diff.	p	Comments
Paulev et al. (1979)	53 (±16)	11 (±4)	12.9 mo (2-37 mo)	32	14	Uln - m(lf)	58.8	55.3	+6	N.S.	Room temperature controlled; no info. on skin temperature. Groups comparable in height, weight, and age range.
						Uln - m(rt)	58.8	53.7	+9	N.S.	
Triebig et al. (1984)	53 actual (22-90) 54 TWA²	<20	11 (1-28)	133	66	Uln - m	58.3	59.2	-2	N.S.	Analysis of subgroupings of Exp. Ss by PbB indicated that effects primarily seen at ≥70 µg/dl.
						Uln - s	52.3	53.1	+2	N.S.	
						Uln - s(d)	45.5	47.5	-4	≤0.05	
						Med - m	46.1	47.1	-2	≤0.05	
Ashby (1980)	60	?	0.5-33	94	94	Uln - m	53.4	55.6	-4	<0.0005	Anomalous pos. correlation observed between ulnar-m NCV and PbB; possibly sig. due to use of multiple t-tests. Skin temperature of Con. Ss < Exp. Ss.
						Med - m	55.9	57.3	-2	<0.01	
						Rad - m	63.9	71.7	-11	<0.0005	
						Per - m	46.1	47.6	-3	<0.005	
						Uln - s	57.5	57.9	-1	N.S.	
	58(±16) (max: <80)	24(±14)	<2	13	13	Uln - m	55.1	58.0	-5	<0.05	Separate analysis of subset of Ss from main study; limited to new employees of <2 yr.
						Med - m	58.4	59.8	-2	N.S.	
						Rad - m	58.1	74.1	-22	<0.005	
						Per - m	46.6	49.9	-7	<0.05	
Singer et al. (1983)	60 (±17)	?	10.6 (0.5-28)	37	26	Med - m	56.1	57.6	-3	0.36	Adjustment of NCVs for age and skin temperature increased statistical significance of effects; for Ss exposed >10 yr, median-m NCV also significantly slower.
				35	24	Med - s	42.9	46.8	-8	0.006	
				25	13	Per - m	49.0	49.2	0	0.87	
				24	20	Sur - s	37.8	42.8	-12	0.0004	
			Overall =	40	31						
Spivey et al. (1980)	[illegible] (±11.9)	22 (±6.2)	≥1	55	31	Uln - m	55.5	56.0	-1	N.S.	Exp. group 2.8 yr older than Con. group. Sig. (<0.05) neg. corr. between ulnar-m NCV and age. Room and skin temperature controlled.
						Uln - sf	45.5	46.9	-3	N.S.	
						Per - m	52.3	51.5	+2	N.S.	
	66 (±12.5)	28 (±8.4)	1-1.5 yr later	55	31	Uln - m	56.2	53.1	+6	0.027	
						Uln - sf	45.3	44.1	+3	N.S.	
						Per - m	50.5	48.9	+3	N.S.	
Sborgia et al. (1983)	63 (±19.0)	24 (±7.4)	7.84	31	35	Med - m	55.4	56.6	-2	N.S.	NCVs corrected for skin temperature. Regression analysis showed no sig. effect of max. or past avg. PbB, but max. ZPP showed sig. association with ulnar NCV.
						Med - sf	50.1	49.7	+1	N.S.	
						Med - s(d)	63.4	62.7	+1	N.S.	
						Med - s(p)	61.2	61.0	0	N.S.	
						Uln - m	55.0	54.5	+1	N.S.	
						Pop - m	49.3	50.0	-1	N.S.	
						Tib - m	48.7	46.4	+5	N.S.	

TABLE 12-1. (continued)

Reference	Mean blood lead, µg/dl Exp. (range/±S.D.)	Mean blood lead, µg/dl Con.	Exposure period, yr. (range/±S.D.)	No. of subjects Exp.	No. of subjects Con.	Nerve*	NCV, m/sec Exp.	NCV, m/sec Con.	% Diff.	p	Comments
Baker et al. (1984)	(60-80)	(0-20)	~2-3 (0-20)	5 (intermediate PbB groups N = 92)	20	Uln - m Uln - s Per - m Sur - s	63.4 55.1 52.6 45.4	62.5 51.8 50.8 48.7	+1 +6 +4 -7	N.S. 0.02 N.S. 0.03	p-values refer to an exposure coefficient (based on 12-mo TWA^2) in a multiple linear regression model allowing for age, height, weight, and skin temperature across all PbB groups.
Feldman et al. (1977)	70 (18-110)	?	?	19	25	Per - m	45.0	54.1	-17	<0.02	No info. on Con. Ss or on ages of Exp. Ss. Skin temperature not controlled.
Englert (1980)	71 (max: 140)	-	?	99	0	Uln - m Uln - sf	58.8 ~47	- -	- -	N.S. N.S.	No sig. correlation between NCV and PbB, but sig. slowing at high ALA-U levels. NCV corrected for age, skin temperature, and body height.
Vasilescu (1973)	72 (27-180)	?	?	50	30	Med - m Uln - m Per - m Rad - m	55.6 56.6 46.1 44.8	57.2 57.3 60.5 49.8	-3 -1 -24 -10	0.05 0.05 0.05 0.05	P values as reported. No info. on skin temperature. Exp. group about 2 yr younger than Con. group.
Melgaard et al. (1976)	78 (38-125)	~19 (2-36)	?	20	12	Per - m Uln - m Uln - s	48.7 55.0 ~57.0	~50 ~58 ~54	-3 -5 +6	? ? ?	Con. values estimated from info. in report. NCVs corrected for skin temperature but not age. Ss exposed to other metals besides Pb.
Catton et al. (1970)	(40-120+)	?	(0.4-13)	19	17	Pop - m Pop - s	49.9 57.1	49.6 56.3	0 +1	N.S. N.S.	Only sig. difference between groups was in ratio of knee and ankle muscle action potentials.
Buchthal & Behse (1979)	(70-140) (max. during preceding year)	?	(0.33-33)	20	?	Med - m Med - s Per - m Sur - s	58.1 63.7 50.1 50.7	63.6 67.1 51.0 54.6	-9 -5 -2 -7	<0.001 <0.01 N.S. <0.001	No histological evidence of abnormality in sural nerve. No info. on how Con. values obtained, but said to be matched for age.

*Med = median; Uln = ulnar; Per = peroneal; Tib = posterior tibial; Sur = sural; Fib = fibular; Pop = lateral popliteal;
m = motor; s = sensory; mx = mixed; sf = slow fibers; lf = left; rt = right; d = distal; p = proximal.

†TWA = time-weighted average.

Rosén et al., 1983; Spivey et al., 1980) has failed to find significant effects with ulnar slow fibers. Nevertheless, the preponderance of effects has been in the negative direction, as reflected in the column of Table 12-1 showing the percent differences in NCVs between lead-exposed and referent groups. Moreover, of the various nerves examined, the most consistently decreased NCVs appear to involve the median motor nerve. Recent experimental work with rats may help explain this finding. Bouldin et al. (1985) found that lead-treated rats showed a greater susceptibility to demyelination in the sciatic nerve (a mixed nerve containing a large number of motor fibers) than in the sural nerve (a sensory nerve). Given the dependence of nerve conduction on the functional integrity of the nerve's myelin sheath, this difference in susceptibility would help explain the variability in results of NCV studies examining different types of nerves and is consistent with the emergence of the median motor nerve as the most prevalent indicator of reduced NCV in the studies listed in Table 12-1.

A problem inherent in nearly all NCV studies has been the lack of experimental manipulation of the presumed cause of lowered conduction velocities. Of interest in this regard is the study of Araki et al. (1980), in which median motor NCVs were measured before and after blood lead levels were lowered through chelation therapy. The investigators found that, depending on a worker's initial NCV and the amount of change in blood lead level achieved through chelation, significantly improved nerve conduction was measured in 7 of 14 lead-exposed workers. For all subjects considered together, the increase in NCV correlated significantly with the decrease in blood lead level ($r = -0.573$, $p < 0.001$). The work of Araki et al. (1980), as well as case studies reported by Feldman et al. (1977), indicate that lead-induced impairment of nerve conduction is reversible, at least in part, by a reduction in blood lead levels through chelation therapy. Although helpful in establishing a causal connection between lead exposure and peripheral nerve function, these studies have not resolved the dispute over whether such effects merely reflect mild, fully reversible impacts of lead (Buchthal and Behse, 1981) or are true early warning signals of progressively more serious neuropathy in otherwise undiagnosed lead intoxication (Feldman et al., 1977; Seppäläinen and Hernberg, 1980).

Taken as a whole, the studies reviewed here indicate the likelihood of NCV effects at blood lead levels below 70 µg/dl, possibly even as low as 30 µg/dl, although further prospective studies are needed to characterize these levels definitively. It is important to note that even though many of the observed changes in NCV may fall within the range of normal variation, these studies show significant effects in groups of subjects, not just individual subjects. Thus, these effects clearly represent departures from normal neurological functioning and should be seriously considered for their potential health significance.

12.4.2.1.3 Other Hypothesized Neurotoxic Effects of Lead in Adults. There are several case reports of previous overexposure to heavy metals, e.g. lead, in amyotrophic lateral sclerosis (ALS) patients and patients dying of motor neuron disease (MND). These reports have led to hypotheses concerning the relationship between such neurotoxic syndromes and lead exposure. Conradi et al. (1976, 1978a,b, 1980), for example, found elevated lead levels in the cerebrospinal fluid of ALS patients as compared with controls. In addition, Kurlander and Patten (1979) found that lead levels in spinal cord anterior horn cells of MND patients were nearly three times that of control subjects and that lead levels correlated with illness durations; despite chelation therapy for about a year, high lead levels remained in their tissue. On the other hand, certain other studies (e.g., Manton and Cook, 1979; Stober et al., 1983) have not found evidence to support an association of lead exposure with ALS. Thus, the evidence for possible pathogenic significance of lead in ALS and motor neuron disease is at best mixed at this time and the issue needs to be further explored by future research.

12.4.2.2 Neurotoxic Effects of Lead Exposure in Children.

12.4.2.2.1 Overt lead intoxication in children. Symptoms of encephalopathy similar to those that occur in adults have been reported to occur in infants and young children (Prendergast, 1910; Oliver, 1911; Blackfan, 1917; McKhann and Vogt, 1926; Giannattasio et al., 1952; Cumings, 1959; Tepper, 1963; Chisolm, 1968), with a markedly higher incidence of severe encephalopathic symptoms and deaths occurring among them than in adults. This may reflect the greater difficulty in recognizing early symptoms in young children, thereby allowing intoxication to proceed to a more severe level before treatment is initiated (Lin-Fu, 1973). In regard to the risk of death in children, the mortality rate for encephalopathy cases was approximately 65 percent prior to the introduction of chelation therapy as standard medical practice (Greengard et al., 1965; National Academy of Sciences, 1972; Niklowitz and Mandybur, 1975). The following mortality rates have been reported for children experiencing lead encephalopathy since the inception of chelation therapy as the standard treatment approach: 39 percent (Ennis and Harrison, 1950); 20-30 percent (Agerty, 1952); 24 percent (Mellins and Jenkins, 1955); 18 percent (Tanis, 1955); and 5 percent (Lewis et al., 1955). These data, and those tabulated more recently (National Academy of Sciences, 1972), indicate that once lead poisoning has progressed to the point of encephalopathy, a life-threatening situation clearly exists and, even with medical intervention, is apt to result in a fatal outcome. Historically there have been three stages of chelation therapy. Between 1946 and 1950, dimercaprol (BAL) was used. From 1950 to 1960, calcium disodium ethylenediaminetetraacetate ($CaNa_2EDTA$) completely replaced BAL. Beginning in 1960, combined therapy with BAL and $CaNa_2EDTA$ (Chisolm, 1968) resulted in a very substantial reduction in mortality.

Determining precise values for lead exposures necessary to produce acute symptoms, such as lethargy, vomiting, irritability, loss of appetite, dizziness, etc., or later neurotoxic sequelae in humans is difficult in view of the usual sparsity of data on environmental lead exposure levels, period(s) of exposure, or body burdens of lead existing prior to manifestation of symptoms. Nevertheless, enough information is available to permit reasonable estimates to be made regarding the range of blood lead levels associated with acute encephalopathic symptoms or death. Available data indicate that lower blood lead levels among children than among adults are associated with acute encephalopathy symptoms. The most extensive compilation of information on a pediatric population is a summarization (National Academy of Sciences, 1972) of data from Chisolm (1962, 1965) and Chisolm and Harrison (1956). This data compilation relates occurrence of acute encephalopathy and death in children in Baltimore to blood lead levels determined by the Baltimore City Health Department (using the dithizone method) between 1930 and 1970. Blood lead levels formerly regarded as "asymptomatic" and other signs of acute lead poisoning were also tabulated. Increased lead absorption in the absence of detected symptoms was observed at blood lead levels ranging from 60 to 300 µg/dl (mean = 105 µg/dl). Acute lead poisoning symptoms other than signs of encephalopathy were observed from approximately 60 to 450 µg/dl (mean = 178 µg/dl). Signs of encephalopathy (hyperirritability, ataxia, convulsions, stupor, and coma) were associated with blood lead levels of approximately 90 to 700 or 800 µg/dl (mean = 330 µg/dl). The distribution of blood lead levels associated with death (mean = 327 µg/dl) was essentially the same as for levels yielding encephalopathy. These data suggest that blood lead levels capable of producing death in children are essentially identical to those associated with acute encephalopathy and that such effects are usually manifested in children starting at blood lead levels of approximately 100 µg/dl. Certain other evidence from scattered medical reports (Gant, 1938; Smith et al., 1938; Bradley et al., 1956; Bradley and Baumgartner, 1958; Cumings, 1959; Rummo et al., 1979), however, suggests that acute encephalopathy in the most highly susceptible children may be associated with blood lead levels in the range of 80-100 µg/dl. These latter reports are evaluated in detail in the 1977 EPA document Air Quality Criteria for Lead (U.S. EPA, 1977).

From the preceding discussion, it can be seen that severity of symptoms varies widely for different adults or children at increasing blood lead levels. Some show irreversible CNS damage or death at blood lead levels around 100 µg/dl, whereas others may not show any of the usual clinical signs of lead intoxication even at blood lead levels in the 100-200 µg/dl or higher range. This diversity of response may be due to the following: (1) individual biological variation in lead uptake or susceptibility to lead effects; (2) changes in blood lead values from the time of initial damaging intoxication; (3) greater tolerance for a gradually accumulating lead burden; (4) other interacting or confounding factors, such as nutritional state or inaccurate determinations of blood lead; or (5) lack of use of blind evaluation procedures on the part of the evaluators. It should also be noted that a continuous gradation of frequency and severity of neurotoxic symptoms extends into the lower ranges of lead exposure.

Morphological findings vary in cases of fatal lead encephalopathy among children (Blackman, 1937; Pentschew, 1965; Popoff et al., 1963). Reported neuropathologic findings are essentially the same for adults and children. On macroscopic examination the brains are often edematous and congested. Microscopically, cerebral edema, altered capillaries (endothelial hypertrophy and hyperplasia), and perivascular glial proliferation often occur. Neuronal damage is variable and may be caused by anoxia. However, in some cases gross and microscopic changes are minimal (Pentschew, 1965). Pentschew (1965) described neuropathology findings for 20 cases of acute lead encephalopathy in infants and young children. The most common finding was activation of intracerebral capillaries characterized by dilation of the capillaries, with swelling of endothelial cells. Diffuse astrocytic proliferation, an early morphological response to increased permeability of the blood-brain barrier, was often present. Concurrent with such alterations, especially evident in the cerebellum, were changes that Pentschew (1965) attributed to hemodynamic disorders, i.e., ischemic changes manifested as cell necrosis, perineuronal incrustations, and loss of neurons, especially in isocortex and basal ganglia.

Attempts have been made to better understand brain changes associated with encephalopathy by studying animal models. Studies of lead intoxication in the CNS of developing rats have shown vasculopathic changes (Pentschew and Garro, 1966), reduced cerebral cortical thickness and reduced number of synapses per neuron (Krigman et al., 1974a), and reduced cerebral axonal size (Krigman et al., 1974b). Biochemical changes in the CNS of lead-treated neonatal rats have also demonstrated reduced lipid brain content but no alterations of neural lipid composition (Krigman et al., 1974a) and a reduced cerebellar DNA content (Michaelson, 1973). In cases of lower level lead exposure, subjectively recognizable neuropathologic features may not occur (Krigman, 1978). Instead there may be subtle changes at the level of the synapse (Silbergeld et al., 1980a) or dendritic field, myelin-axon relations, and organization of synaptic patterns (Krigman, 1978). Since the nervous system is a dynamic structure rather than a static one, it undergoes compensatory changes (Norton and Culver, 1977), maturation and aging (Sotelo and Palay, 1971), and structural changes in response to environmental stimuli (Coss and Glohus, 1978). Thus, whereas massive structural damage in many cases of acute encephalopathy would be expected to cause lasting neurotoxic sequelae, some other CNS effects due to severe early lead insult might be reversible or compensated for, depending upon age and duration of toxic exposure. This raises the question of whether effects of early overt lead intoxication are reversible beyond the initial intoxication or continue to persist.

In cases of severe or prolonged nonfatal episodes of lead encephalopathy, there occur neurological sequelae qualitatively similar to those often seen following traumatic or infectious cerebral injury, with permanent sequelae being more common in children than in adults (Mellins and Jenkins, 1955; Chisolm, 1962, 1968). The most severe sequelae in children are cortical atrophy, hydrocephalus, convulsive seizures, and severe mental retardation (Mellins and Jenkins, 1955; Perlstein and Attala, 1966; Chisolm, 1968). Children who recover from acute lead encephalopathy but are re-exposed to lead almost invariably show evidence of permanent central nervous system damage (Chisolm and Harrison, 1956). Even if further lead exposure is minimized, 25-50 percent show severe permanent sequelae, such as seizure disorders, blindness, and hemiparesis (Chisolm and Barltrop, 1979).

Lasting neurotoxic sequelae of overt lead intoxication in children in the absence of acute encephalopathy have also been reported. Byers and Lord (1943), for example, reported that 19 out of 20 children with previous lead poisoning later made unsatisfactory progress in school, presumably due to sensorimotor deficits, short attention span, and behavioral disorders. These latter types of effects have since been confirmed in children with known high exposures to lead, but without a history of life-threatening forms of acute encephalopathy (Chisolm and Harrison, 1956; Cohen and Ahrens, 1959; Kline, 1960). Perlstein and Attala (1966) also reported neurological sequelae in 140 of 386 children (37 percent) following lead poisoning without encephalopathy. Such sequelae included mental retardation, seizures, cerebral palsy, optic atrophy, and visual-perceptual problems in some children with minimal intellectual impairment. The severity of sequelae was related to severity of earlier observed symptoms. For 9 percent of those children who appeared to be without severe symptoms at the time of diagnosis of overt lead poisoning, mental retardation was observed upon later follow-up. Since no control group was included in their study, one may question whether the neurological effects observed by Perlstein and Attala (1966) were persisting effects of earlier overt lead intoxication without encephalopathy; however, it is extremely unlikely that 37 percent of any randomly selected control group from the general pediatric population would exhibit the types of neurological problems observed by Perlstein and Attala (1966).

Numerous studies (Cohen et al., 1976; Fejerman et al., 1973; Pueschel et al., 1972; Sachs et al., 1978, 1979, 1982) suggest that, in the absence of encephalopathy, chelation therapy may ameliorate the persistence of neurotoxic effects of overt lead poisoning (especially cognitive, perceptual, and behavioral deficits). On the other hand, one recent study found a residual effect on fine motor performance even after chelation (Kirkconnell and Hicks, 1980).

In summary, pertinent literature definitively demonstrates that lead poisoning with encephalopathy results in a greatly increased incidence of permanent neurological and cognitive impairments. Also, several studies further indicate that children with symptomatic lead poisoning in the absence of encephalopathy also show a later increased incidence of neurological and behavioral impairments.

12.4.2.2.2 Non-overt lead intoxication in children. In addition to neurotoxic effects associated with overt lead intoxication in children, substantial evidence indicates that lead exposures not leading to overt lead intoxication in children can induce neurological dysfunctions. This issue has attracted much attention and generated considerable controversy during the past 10-15 years. However, the evidence for and against the occurrence of significant neurotoxic deficits at relatively low levels of lead exposure has been quite mixed and largely interpretable only after a thorough critical evaluation of methods employed in the various important studies on the subject. Based on five of the criteria listed earlier (i.e., adequate markers of exposure to lead, sensitive measures, appropriate subject selection, control of confounding covariates, and appropriate statistical analysis), the population studies summarized in Table 12-2 were conducted rigorously enough to warrant at least some consideration here. Even so, no epidemiological study is completely flawless and, therefore, overall interpretation of such findings must be based on evaluation of the following: (1) the internal consistency and quality of each study; (2) the consistency of results obtained across independently conducted studies; and (3) the plausibility of results in view of other available information.

Rutter (1980) has classified studies evaluating neurobehavioral effects of lead exposure in non-overtly lead intoxicated children according to several types, including four categories reviewed below: (1) clinic-type studies of children thought to be at risk because of high lead levels; (2) other studies of children drawn from general (typically urban or suburban) pediatric populations; (3) samples of children living more specifically in close proximity to lead emitting smelters; and (4) studies of mentally retarded or behaviorally deviant children. Major attention is accorded here to studies falling under the first three categories. A final section discusses some initial results beginning to emerge from long-term prospective studies, which attempt to relate effects on early neuropsychological development and later neuropsychologic functioning to lead exposure histories for children documented back to birth or even prenatally.

12.4.2.2.2.1 Clinic-type studies of children with high lead levels. The clinic-type studies are generally typified by evaluation of children with relatively high lead body burdens as identified through lead screening programs or other large-scale programs focusing on mother-infant health relationships and early childhood development.

De la Burde and Choate (1972) observed neurological dysfunctions, fine motor dysfunction, impaired concept formation, and altered behavioral profiles in 70 preschool children exhibiting pica and elevated blood lead levels (in all cases above 30 µg/dl; mean = 59 µg/dl) in comparison with matched control subjects not engaging in pica. Subjects were drawn from the

TABLE 12-2. SUMMARY OF STUDIES ON NEUROBEHAVIORAL FUNCTIONS OF LEAD-EXPOSED CHILDREN[a]

Reference	Population studied	N/group	Age at testing, yr (range)	Blood lead, µg/dl (range or ±S.D.)	Psychometric tests employed	Summary of results					Levels of significance[b]
Clinic-type studies of children with high lead levels											
							C	Pb			
de la Burde and Choate (1972)	Inner city (Richmond, VA)	C = 72	4.0	?^{c}	IQ (Stanford-	Score:	94	89			
		Pb = 70	4.0	58 (30-100)^d	Binet)	% subnormal:^{e}	10	25			<0.05
					Fine motor		26	45			<0.05
					Gross motor		7	16			N.S.
					Concept formation		10	15			N.S.
					Behavior profile		10	30			<0.01
de la Burde and Choate (1975)	Follow-up same subjects	C = 67	7-8	PbT: 112 µg/g	WISC Full Scale	Score:	90	87			
		Pb = 70	7-8	202 µg/g	IQ	% subnormal:^{e}	6	24			0.01
					Verbal IQ		9	18			N.S.
					Performance IQ		13	24			N.S.
					Bender-Gestalt		27	49			0.01
					Reading		7	12			N.S.
					Spelling		11	16			N.S.
					Arithmetic		7	12			N.S.
					Goodenough-Harris draw test		1	13			0.02
					Auditory vocal assoc.		13	31			0.01
					Tactile recognition		3	15			0.05
					Behavior profile		3	25			0.001
Rummo (1974); Rummo et al. (1979)	Inner city (Providence, RI)	C = 45	5.8	23 (±8)	McCarthy Scales:	C	Pb_1	Pb_2	Pb_3		
		Pb_1 = 15	5.6	61 (±7)	Gen. cognitive	93	94	88	77		<0.01
		Pb_2 = 20	5.6 (4-8)	68 (±13)	Verbal	46	46	44	37		<0.05
		Pb_3 = 10	5.3	88 (±41)	Perceptual	48	49	46	38		<0.05
					Quantitative	45	44	41	35		<0.01
					Memory	47	46	43	36		<0.01
					Motor	52	52	50	40		<0.01
					Parent ratings	8	10	10	18		<0.01
					Neurologic exam	7/12 measures sig. different					
Kotok (1972)	Inner city (New Haven, CT)	C = 25	2.7 (1.1-5.5)	38 (20-55)	Denver Develop-						
		Pb = 24	2.8 (1.0-5.8)	81 (58-137)	mental:	Norm	C	Pb			
					Gross motor	1.00	1.02	1.06			N.S.^{f}
					Fine motor	1.00^f	0.82	0.81^f			<0.01^f
					Language	1.00^f	0.82	0.73^f			<0.01^f
Kotok et al. (1977)	Inner city (Rochester, NY)	C = 36	3.6 (1.9-5.6)	28 (11-40)	IQ Equivalent:		C	Pb			
		Pb = 31	3.6 (1.7-5.4)	80 (61-200)	Social		126	124			>0.10
					Spatial		101	92			<0.10
					Spoken vocal.		93	92			>0.10
					Info-comprehension		96	94			>0.10
					Visual attention		93	90			>0.10
					Auditory memory		100	93			>0.10

TABLE 12-2. (continued)

Reference	Population studied	N/group	Age at testing, yr (range)	Blood lead, µg/dl (range or ±S.D.)	Psychometric tests employed	Summary of results		Levels of significance^{b}
Perino and Ernhart (1974)^{f}	Inner city (New York, NY)	Low Pb = 50	3-6	10-30	McCarthy Scales:	Low	Mod.	
		Mod. Pb = 30	3-6	40-70	Gen. cognitive	90	80	<0.01
					Verbal	44	39	<0.05
					Perceptual	44	37	<0.05
					Quantitative	48	44	N.S.
					Memory	45	42	N.S.
					Motor	46	42	N.S.
Ernhart et al. (1981)^{f}	Follow-up same subjects	Low Pb = 31	8-13	21 (±4)^{g}	McCarthy Scales:	Low	Mod.	
		Mod. Pb = 32		32 (±5)	Gen. cognitive	94	82	<0.05
					Verbal	48	41	<0.05
					Perceptual	43	40	N.S.
					Quantitative	43	38	N.S.
					Memory	44	39	N.S.
					Motor	49	46	<0.05
					Reading tests	Not Reported		N.S.
					Conners teacher ratings	Not Reported		N.S.
					Various experimental tests	Not Reported		N.S.
General Population Studies						C	Pb	
Needleman et al. (1979)^{h}	Urban (Boston, MA)	C = 100	7	PbT: <10 ppm^{i}	WISC Full Scale IQ	106.6	102.1	0.03
		Pb = 58	7	>20 ppm	Verbal IQ	103.9	99.3	0.06
					Performance IQ	108.7	104.9	0.12
					Seashore Rhythm Test	21.6	19.4	0.002
					Token Test	24.8	23.6	0.09
					Sentence Repetition Test	12.6	11.3	0.04
					Delayed Reaction Time	C > Pb on 3/4 blocks		<0.01
					Teacher Ratings	9.5	8.2	0.02
McBride et al. (1982)	Urban/suburban (Sydney, Australia)	Low Pb = >100	4/5	2-9 µg/dl	Peabody Picture	Low	Mod.	
		Mod. Pb = >100	4/5	19-29 g/dl	Vocab. Test	~105	~104	N.S.
					Fine Motor Tracking	C > Pb 1/4 comparisons		<0.05
					Pegboard	~20	~20	N.S.
					Tapping Test	~30	~31	N.S.
					Beam Walk	~5	~4	N.S.
					Standing Balance	C > Pb 1/4 comparisons		<0.05
					Rutter Activity Scale	~1.9	~2.1	N.S.

TABLE 12-2. (continued)

Reference	Population studied	N/group	Age at testing, yr (range)	Blood lead, μg/dl (range or ±S.D.)	Psychometric tests employed	Summary of results				Levels of significance[b]
						Group: 1	2	3	4	
Yule et al. (1981)	Urban (London, England)	Group 1 = 34	9	8.8[j] (7-10)	WISC-R Full Scale IQ	103	103	96	96	0.027
		Group 2 = 48	9 (6-12)	11.6 (11-12)	Verbal IQ	101	101	95	94	0.043
		Group 3 = 49	8	14.5 (13-16)	Performance IQ	106	103	98	99	0.102
		Group 4 = 35	8	19.6 (17-32)	Vernon Spelling Test	104	98	92	89	0.001
					Vernon Math Test	97	97	95	95	N.S.
					Neale Reading Accuracy[k]	121	110	96	89	0.001
					Neale Reading Compre.[k]	117	110	95	88	0.001
Yule et al. (1984)	Same subjects in Yule et al. (1981)	Same	Same	Same	Needleman Teacher Ratings	1.53	1.54	2.45	2.63	0.096
						(4/11 items sig. different)				
					Conners Teacher Ratings	0.26		0.37		0.04
						(3/4 factors sig. at p <0.05)				
					Rutter Teacher Ratings, including "Overactivity" factor	(2/26 items sig. at p ≤0.05) (5/26 items differ at 0.05<p<0.10)				
						6%	4%	20%	17%	0.04
							Low	High		
Lansdown et al. (1986)	Urban (London, England)	Low = 80	9	7-12	WISC-R Full Scale		107	105		N.S.
		High = 82	9	13-24	Verbal IQ		104	103		N.S.
					Performance IQ		108	106		N.S.
					Neale Reading Accuracy		115	112		N.S.
					Neale Reading Comprehension		113	109		N.S.
					Vernon Spelling		101	99		N.S.
					Vernon Math		100	99		N.S.
						Low	Med	High		
Smith et al. (1983)	Urban (London, England)	High = 155	6,7	PbT ≥ 8.0	WISC-R Full Scale	107	105	105		N.S.
		Med = 103	6,7	PbT = 5-5.5	Verbal IQ	105	103	103		N.S.
		Low = 145	6,7	PbT < 2.5	Performance IQ	108	106	106		N.S.
				(All in μg/g)	Word Reading Test	45	42	40		N.S.
				$\bar{X}$ PbB = 13.1 μg/dl	Seashore Rhythm Test	21	20	20		N.S.
					Visual Sequential Memory	20	19	20		N.S.
					Sentence Memory	9	9	9		N.S.
					Shape Copying	14	14	14		N.S.
					Mathematics	16	15	15		N.S.
					Mean Visual RT (secs)	0.37	0.37	0.39		N.S.
					Conners Teacher Ratings	11	11	13		N.S.

TABLE 12-2. (continued)

Reference	Population studied	N/group	Age at testing, yr (range)	Blood lead, µg/dl (range or ±S.D.)	Psychometric tests employed	Summary of results	Levels of significance[b]
Harvey et al. (1983, 1984)	Urban (Birmingham, England)	189	2.5	15.5 (6-30)	British Ability Scales	Regression F Ratio[l]	
					Naming	<1	N.S.
					Recall	1.26	N.S.
					Comprehension	<1	N.S.
					Recognition	<1	N.S.
					IQ	<1	N.S.
					Stanford-Binet Items		
					Shapes	<1	N.S.
					Blocks	2.34	N.S.
					Beads	2.46	N.S.
					Playroom Activity	?	N.S.
Silva et al. (1986b)	Urban (Dunedin, New Zealand)	579	11	11.1 (4-50)	WISC-R Full Scale IQ	r = -0.06	N.S.
					Performance IQ	= -0.03	N.S.
					Verbal	= -0.05	N.S.
					Rutter Behavior Rating	R^2 Incrs. by Pb	
					Parent	0.21 1.38	0.003
					Teacher	0.10 1.23	0.008
					Inattention Rating		
					Parent	0.24 0.26	N.S.
					Teacher	0.25 1.51	0.001
					Hyperactivity Rating		
					Parent	0.12 1.01	0.015
					Teacher	0.12 0.82	0.028
					Burt Reading Test	0.43 0.28	N.S.
Schroeder et al. (1985)	Rural/urban (Wake County, North Carolina)	104	<2.5 or >2.5 (0.8-6.5)	6-59	Bayley MDI or Stanford-Binet	Regression analysis of sources for IQ effect:	
						Lead: F = 7.689	<0.01
						SES: F = 20.159	<0.001
	Follow-up same subjects	50	6-12	≤30	Stanford-Binet	Lead: F <1	N.S.
Schroeder and Hawk (1986)	Rural/urban (Lenoir and Hanover Counties, North Carolina	75	2	21 (6-47)	Stanford-Binet	Regression analysis of IQ against:	
						Current PbB: F = 12.31	<0.0008
						Max. PbB: F = 10.55	<0.0018
						Mean PbB: F = 10.08	<0.002
Smelter Area Studies						C Pb	
Landrigan et al. (1975)	Smelter area (El Paso, TX)	C = 78 Pb = 46	9.3 (3.8-15.9) 8.3	<40 40-68	WISC Full Scale IQ[m]	93 89	N.S.
					WPPSI Full Scale IQ[n]	91 86	N.S.
					WISC + WPPSI Combined	93 88	N.S.
					WISC + WPPSI Subscales	C > Pb on 13/14 scales 7/14 scales sig. different	<0.05
					Neurologic testing	C > Pb on 7/8 tests 1/8 tests sig. different	<0.01

TABLE 12-2. (continued)

Reference	Population studied	N/group	Age at testing, yr (range)	Blood lead, µg/dl (range or ±S.D.)	Psychometric tests employed	Summary of results		Levels of significance[b]
McNeil and Ptasnik (1975)	Smelter area (El Paso, TX)	C = 37 Pb = 101	9 9 (1.8-18)	29 (14-39) 58 (40-93)		C	Pb	
					McCarthy General Cognitive	82	81	N.S.
					WISC-WAIS Full Scale IQ	89	87	N.S.
					Oseretsky Motor Level	101	97	N.S.
					California Personality	C > Pb, 6/10 items		<0.05
					Frostig Perceptual Quotient	100	103	N.S.
					Finger-Thumb Apposition	27	29	N.S.
Ratcliffe (1977)	Smelter area (Manchester, England)	Mod. Pb = 23 Hi Pb = 24	4.7 (4.1-5.6) 4.8 (4.2-5.4)	28 (18-35) 44 (36-64)		Mod.	High	
					Griffiths Mental Dev.	108	102	N.S.
					Frostig Visual Perception	14.3	11.8	N.S.
					Pegboard Test			
					Dominant hand	17.5	17.3	N.S.
					Nondominant hand	19.5	19.8	N.S.
Winneke et al. (1982a)	Smelter area (Duisburg, FRG)	C = 26 Pb = 26	8 8	PbT = 2.4 ppm[h] PbT = 9.2 ppm No PbB		C	Pb	
					German WISC Full Scale	122	117	N.S.
					Verbal IQ	130	124	N.S.
					Performance IQ	130	123	N.S.
					Bender Gestalt Test	17.2	19.6	<0.05
					Standard Neurological Tests	2.7	7.2	N.S.
					Conners Teacher Ratings	?	?	N.S.
Winneke et al. (1983)	Smelter area (Stolburg, FRG)	89	9.4	PbT: 6.16 ppm[h] PbB: 14.3 µg/dl	German WISC	% Variance Due to PbT		
					Full Scale IQ	-0.0		N.S.
					Verbal IQ	-0.5		N.S.
					Performance IQ	+0.6		N.S.
					Bender Gestalt Test	+2.1		<0.05
					Standard Neurological Tests	+1.2		N.S.
					Conners Teacher Ratings	0.4-1.3		N.S.
					Wiener Reaction Performance	+2.0		N.S.

TABLE 12-2. (continued)

Reference	Population studied	N/group	Age at testing, yr (range)	Blood lead, μg/dl (range or ±S.D.)	Psychometric tests employed	Summary of results	Levels of significance[b]
Winneke et al. (1984)	Smelter area (Nordenham, FRG)	122	6.5	8.2 (4.4-22.8)	German WISC	% Variance Due to Pb	
					Short form	-0.3	N.S.
					Verbal IQ	+0.3	N.S.
					Performance	-2.4	N.S.
					Bender Gestalt Test	+0.5	N.S.
					Signalled Reaction Time		
					Short	+0.1	N.S.
					Long	-0.2	N.S.
					Wiener Reaction Time		
					Easy	+4.3	<0.05
					Difficult	+11.0	<0.01

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[a] Abbreviations: C = control subjects; Pb = lead-exposed subjects; MDI = mental development index; N.S. = nonsignificant ($p > 0.05$); PbT = tooth lead; WISC = Wechsler Intelligence Scale for Children; WPPSI = Wechsler Preschool and Primary Scale of Intelligence; RT = Reaction Time.

[b] Significance levels are those found after partialing out confounding covariates.

[c] Urinary coproporphyrin levels were not elevated.

[d] Some with positive radiologic findings, suggesting earlier exposure in excess of 40-60 μg/dl.

[e] Percent of each group scoring "borderline," "suspect," "defective," or "abnormal."

[f] Reanalysis of data by Ernhart correcting for methodological problems in earlier published analyses described here mainly did not substantiate significant differences between control and Pb-exposed children indicated in last two columns to the right (see chapter text).

[g] Dentine levels not reported for statistical reasons.

[h] Reanalyses of Needleman data correcting for methodological problems in earlier published analyses confirmed significant differences between study groups indicated in last two righthand columns for WISC IQ test results (see chapter text).

[i] Main measure was dentine lead (PbT).

[j] Blood lead levels taken 9-12 months prior to testing; none above 33 μg/dl.

[k] Data not corrected for age.

[l] This F ratio is result of testing the difference in sums of squares for two regression equations (one including and one excluding blood lead level as an independent variable) against the residual mean square of the equation including blood lead.

[m] Used for children over 5 years of age.

[n] Used for children under 5 years of age.

Collaborative Study of Cerebral Palsy, Mental Retardation, and Other Neurologic Disorders of Infancy and Childhood (Broman et al., 1975), which was conducted in Richmond, Virginia, and had a total population of 3400 mothers. All mothers in this group were followed throughout pregnancy and all children were postnatally evaluated by regular pediatric neurologic examinations, psychological testing, and medical interviews. All children subject to prenatal, perinatal, and early postnatal insults were excluded from the study, and all had to have normal neurologic examinations and Bayley tests at eight to nine months of age. These are important points which add value to the study. It is unfortunate that blood lead data were not regularly obtained; however, at the time of the study in the late 1960s, 10-20 ml of venous blood was required for a blood lead determination and such samples usually had to be obtained by either jugular or femoral puncture. The other control features (housing location and repeated urinary coproporphyrin tests) would be considered the state of the art for such a study at the time that it was carried out.

In a follow-up study on the same children (at 7-8 years old), de la Burde and Choate (1975) reported continuing CNS impairment in the lead-exposed group as assessed by a variety of psychological and neurological tests. In addition, seven times as many lead-exposed children were repeating grades in school or being referred to the school psychologist, despite many of their blood lead levels having by then dropped significantly from the initial study. In general, the de la Burde and Choate (1972, 1975) studies appear to be methodologically sound, having many features that strengthen the case for the validity of their findings. For example, there were appreciable numbers of children (67 lead-exposed and 70 controls) whose blood lead values were obtained in preschool years and who were old enough (7 years) during the follow-up study to cooperate adequately for reliable psychological testing. The psychometric tests employed were well standardized and acceptable as sensitive indicators of neurobehavioral dysfunction, and the testing was carried out in a blind fashion (i.e., without the evaluators knowing which were control or lead-exposed subjects).

The de la Burde and Choate (1972, 1975) studies might be criticized on several points, but none provide sufficient grounds for rejecting their results. One difficulty is that blood lead values were not determined for control subjects in the initial study; but the lack of history of pica for paint and plaster, as well as tooth lead analyses done later for the follow-up study, render it improbable that appreciable numbers of lead-exposed subjects might have been wrongly assigned to the control group. Subjects in the control group did have a history of pica, but not for paint. Also, results indicating no measurable coproporphyrins in the urine of control subjects at the time of initial testing further confirm proper assignment of those children to the nonexposed control group. A second point of criticism is the use of multiple chi-square statistical analyses, but the fact that the control subjects did significantly better on virtually every measure makes it unlikely that all of the observed effects were due to chance alone. One last problem concerns ambiguities in subject selection which complicate interpretation of the results obtained. Because the lead-exposed group included children with blood lead levels of 40-100 µg/dl, or of at least 30 µg/dl with "positive radiographic findings of lead lines in the long bones, metallic deposits in the intestines, or both," observed deficits might be attributed to blood lead levels as low as 30 µg/dl. Other evidence (Betts et al., 1973), however, suggests that such a simple interpretation is probably not accurate. That is, the Betts et al. (1973) study indicates that lead lines are usually seen only if blood levels exceed 60 µg/dl for most children at some time during exposure, although some (about 25 percent) may show lead lines at blood lead levels of 40-60 µg/dl. In view of this, the de la Burde and Choate results can probably be most reasonably interpreted as showing persisting neurobehavioral deficits at blood lead levels of 40-60 µg/dl or higher.

In another clinic-type child study, Rummo (1974) and Rummo et al. (1979) found significant neurobehavioral deficits (hyperactivity, lower scores on McCarthy scales of cognitive function, etc.) among Providence, Rhode Island, inner-city children who had previously experienced high levels of lead exposure that had produced acute lead encephalopathy. Mean maximum blood lead levels recorded for those children at the time of encephalopathy were 88 ± 40 µg/dl. However, children with moderate blood lead elevation but not manifesting symptoms of encephalopathy were not significantly different (at $p < 0.05$) from controls on any measure of cognitive functioning, psychomotor performance, or hyperactivity. Still, when the data from the Rummo et al. (1979) study for performance on the McCarthy General Cognitive Index or several McCarthy Subscales are compared (see Table 12-2), the scores for long-term moderate-exposure subjects consistently fall below those for control subjects and lie between the latter and the encephalopathy group scores. Thus, it appears that long-term moderate lead exposure, in fact, likely exerted dose-related neurobehavioral effects. The overall dose-response trend might have been shown to be statistically significant if other types of analyses were used, if larger samples were assessed, or if control subjects were restricted to blood lead values below 10 µg/dl. However, control for confounding variables in the different exposure groups would also have to be considered. Note that (1) the maximum blood lead levels for the short-term and long-term exposure subjects were all greater than 40 µg/dl (means = 61 ± 7 and 68 ± 13 µg/dl, respectively), whereas control subjects all had blood lead levels below 40 µg/dl (mean = 23 ± 8 µg/dl), and (2) the control and lead-exposed subjects were inner-city children well matched for socioeconomic background, parental education levels, incidence of pica, and other pertinent factors, but parental IQ was not ascertained and controlled for as a potentially confounding variable.

A somewhat similar pattern of results emerged from a study by Kotok et al. (1977) in which 36 Rochester, New York, control-group children with blood lead levels less than 40 µg/dl were compared with 31 children having distinctly elevated blood lead levels (61-200 µg/dl) but no classical lead intoxication symptoms. Both groups were well matched on important background factors, notably including their propensity to exhibit pica. Again, no clearly statistically significant differences between the two groups were found on numerous tests of cognitive and sensory functions. However, mean scores of control-group children were consistently higher than those of the lead-exposed group for all six of the ability classes listed, even though the control group included children that had notably elevated blood lead values by current medical standards. Kotok (1972) had reported earlier that developmental deficiencies (using the comparatively insensitive Denver Development Screening test) in a group of children having elevated lead levels (58-137 µg/dl) were identical to those in a control group similar in age, sex, race, environment, neonatal condition, and presence of pica, but whose blood lead levels were lower (20-55 µg/dl). Children in the lead-exposed group, however, had blood lead levels as high as 137 µg/dl, whereas some control children had blood lead levels as high as 55 µg/dl. Thus, the study essentially compared two groups with different degrees of markedly elevated lead exposure rather than one of lead-exposed versus nonexposed control children.

Perino and Ernhart (1974) reported a relationship between neurobehavioral deficits and blood lead levels ranging from 40 to 70 µg/dl in a group of 80 inner-city preschool black children, based on the results of a cross-sectional study including children detected as having elevated lead levels via the New York City lead screening program. One key result reported was that the high-lead children had McCarthy Scale IQ scores markedly lower than those of the low-lead group (mean IQ = 80 versus 90, respectively). Also, the normal correlation of 0.52 between parents' intelligence and that of their offspring was found to be reduced to only 0.10 in the lead-exposed group, presumably because of the influence of another factor (lead) that interfered with the normal intellectual development of the lead-exposed children. Another possible explanation for the reported results, however, might be differences in the educational backgrounds of parents of the control subjects when compared with lead-exposed subjects, because parental education level was found to be significantly negatively related to blood lead levels of the children participating in the Perino and Ernhart (1974) study. The importance of this point lies in the fact that several other studies (McCall et al., 1972; Elardo et al., 1975; Ivanans, 1975) have demonstrated that higher parental education levels are associated with more rapid development and higher intelligence quotients (IQs) for their children.

Ernhart et al. (1981) were able to trace and carry out follow-up evaluations on 63 of the 80 preschool children of the Perino and Ernhart (1974) study once they reached school age, using the McCarthy IQ scales, various reading achievement tests, the Bender-Gestalt test, the

Draw-A-Child test, and the Conners Teacher's Questionnaire for hyperactivity. The children's blood lead levels were reported to be significantly correlated with FEP ($r = 0.51$) and dentine lead levels ($r = 0.43$), but mean blood lead levels of the moderately elevated group had decreased after five years. When control variables of sex and parental IQ were extracted by multivariate analyses, the observed differences were reported to be greatly reduced but remained statistically significant for three of seven tests on the McCarthy scales in relation to concurrently measured blood lead levels but not in relation to the earlier blood lead levels or dentine lead levels for the same children. This led Ernhart et al. (1981) to reinterpret their 1974 (Perino and Ernhart, 1974) IQ results (in which they had not controlled for parental education) as either not likely being due to lead or, if due to lead, then representing only minimal effects on intelligence.

The Perino and Ernhart (1974) and Ernhart et al. (1981) studies were evaluated by an expert committee convened by EPA in March, 1983. The committee reported (Expert Committee on Pediatric Neurobehavioral Evaluations, 1983) certain methodological problems associated with the analyses published by Perino and Ernhart (1974) and Ernhart et al. (1981). The committee further recommended that the Ernhart data set be reanalyzed to deal with the methodological problems. Results of reanalyses of the data have been submitted by Ernhart (1983, 1984; Ernhart et al., 1985). Reanalysis of relationships between preschool-age children's blood lead levels and concurrently obtained McCarthy Scales scores (which included corrections of errors made in the earlier, published analyses for certain data calculations and degrees of freedom used to determine statistical significance) revealed no statistically significant differences (at $p < 0.05$) due to lead; however, lower scores for the higher lead exposure group on the General Cognitive Index (GCI) did approach significance at $p < 0.09$. Also, reanalysis of relationships between preschool lead levels and 5-year later school-age outcome variables yielded no indication of persisting lead effects in terms of reading test results or scores on the McCarthy GCI or most of the McCarthy Subscales (except for a p-value of 0.10 obtained for Verbal Index scores). The reanalysis of relationships between school-age blood lead levels (newly corrected for hematocrit variation effects) and concurrent reading test and McCarthy Scales scores only found significant differences attributable to lead for lower McCarthy Verbal Index scores ($p < 0.036$ with a "deviant case" included in the analysis and $p < 0.07$ with the case excluded). Similar results were obtained with a different analysis employing a "lead construct index" as a measure of lead exposure which combined preschool and school-age blood lead levels and free erythrocyte protoporphyrin levels. Based on these results, Ernhart et al. (1985) concluded that "the reanalyses provide no reasonable support for an interpretation of lead effects in these data." However, she also noted that it is recognized that there was a certain level of unreliability in the measures used and that the sample size limited the power of the statistical analyses. Given such limitations and extensive attention accorded to statistical control of potentially confounding variables in the reanalyses, it is notable that an association between lead and lower Verbal Index scores was nevertheless observed across several of the analyses (at p values ranging from <0.04 to 0.10) and that an association between preschool lead levels and General Cognitive Index scores approached significance at p <0.09. These observations (possibly due to chance alone from among the large number of statistical analyses conducted) do not provide much evidence for associations between neuropsychologic deficits and lead exposures at the levels experienced by children in the Ernhart study population; conversely, however, results of the reanalyses do not allow for a definitive conclusion of "no-effect," either (as noted by Ernhart, 1983).

Other investigators (Shapiro and Marecek, 1984; Marecek et al., 1983) studied relationships between lead exposures and psychometric testing outcomes among black children who had been members of the Philadelphia Collaborative Perinatal Project (CPP), which included mainly families of low socioeconomic status. From among a large target sample of eligible children (those young enough to have deciduous teeth and no past history of head trauma, mental retardation, or lead poisoning) invited to participate in the study (2,568 letters of invitation were mailed), 199 families enrolled their children. Each child was scheduled for neuropsychologic testing immediately following the loss of a tooth; primary and/or circumpulpal dentine lead levels from shed deciduous teeth (mainly molars) were employed to provide an index of lead exposure for the 188 children (aged 10.6 to 14.7 yr; $\bar{X}$ = 11.8 yr) who underwent neuropsychologic testing. Data on socioeconomic status and several other potentially confounding variables were obtained from CPP records, and IQ scores were obtained for the parents of a subset of the children studied. Data analyses (hierarchical multiple regression analyses) first evaluated relationships between dentine lead exposure indices and test scores obtained several years earlier (at age 7 yr) on the Bender-Gestalt, Wechsler Intelligence Scale for Children (WISC) subtests, and certain other neuropsychologic tests; analyses were also performed using dentine lead data and results from concurrently administered psychometric tests. For the age-seven tests, significant associations were reported between dentine lead and performance IQ scores, but not for WISC verbal IQ scores. Similarly, significant relationships (at p <0.05) were reported between dentine lead values and concurrently obtained test results for performance abilities on the Bender-Gestalt, WISC, and other tests but not for verbal abilities. This study, while qualitatively suggesting lead may affect performance abilities, suffers from several methodological problems, including inadequate control for sampling bias, retrospective estimation of age-seven lead exposure levels, poor control of covarying social factors, and inadequate control for parental IQ influences for all children studied.

Odenbro et al. (1983) studied psychological development of children (aged 3-6 yr) seen in Chicago Department of Health Clinics (August, 1976 - February, 1977), evaluating scores on the

Denver Developmental Screening test and two subtests of the Wechsler Preschool and Primary Scale of Intelligence (WPPSI) in relation to blood lead levels obtained by repeated sampling during the three previous years. A significant correlation ($r = -0.435$, $p < 0.001$) was reported between perceptual visual-motor ability and mean blood lead levels. Statistically significant ($p < 0.005$) deficits in verbal productivity and perceptual visual motor performance (measured by the WPPSI) were found for groups of children with mean blood lead levels of 30-40 μg/dl and 40-60 μg/dl versus control children with mean blood lead levels <25 μg/dl, using two-tailed Student's t-tests. On the other hand, significant associations ($p < 0.05$) between blood lead levels and developmental retardations in language and fine-motor functions were found only for the 40-60 μg/dl group, using the Denver Development Screening test and chi-square analyses. These results are most clearly suggestive of neuropsychologic deficits being associated with blood lead levels of 40-60 μg/dl in preschool children. However, parental IQs were not measured and questions can be raised regarding the adequacy of the statistical analyses employed, especially in regard to lack of use of multivariate analyses that sufficiently control for confounding covariates such as parental education and socioeconomic status.

In another study (Molina et al., 1983), high-risk children from families making lead-glazed pottery in a Mexican village were evaluated for lead-associated neuropsychologic deficits, using an appropriately adapted Spanish language version of the revised WISC (WISC-R) test and the Bender-Gestalt test. Test results for 33 high-lead children ($\bar{X}$ age: 10 yr, 7 mo ± 2 yr, 7 mo) randomly selected from 64 school children with blood lead levels above 40 μg/dl ($\bar{X}$: 63.4 ± 15.8 μg/dl) were compared with those for 30 lower lead children ($\bar{X}$ age: 10 yr, 2 mo ± 2 yr, 6 mo) with blood lead levels below 40 μg/dl ($\bar{X}$: 26.3 ± 8.0 μg/dl), using the two-tailed Student's t-test and the Mann-Whitney U test. The high-lead children were found to have significantly lower WISC-R full-scale IQ ($p < 0.01$), verbal IQ ($p < 0.01$), and performance IQ ($p < 0.025$) than did the low-lead control children, who were drawn from among the same low socioeconomic class families as the high-lead children. A significant negative linear correlation was also observed for the same categories of test scores among the high-lead children, but not for such scores among the low-lead children. These results, highly suggestive of lead-related neuropsychologic deficits in children with blood lead values over 40 μg/dl, must be viewed with caution in light of the failure to include parental IQ levels and the lack of multivariate statistical analyses that explicitly controlled for age, sex, or other confounding factors.

In summary, the studies reviewed above generally found that high-risk lead-exposure groups did more poorly on IQ or other types of psychometric tests than referent control groups with distinctly lower lead exposures. It is true that many of the studies did not control for important confounding variables or, when such were taken into account, differences between lead-exposed and control subjects were reduced and, at times, often no longer statistically significant. Still, the consistency of finding lower IQ values and other types of neuropsychologic deficits among at-risk higher lead exposure children across most of the studies reviewed lends credence to cognitive deficits occurring in apparently asymptomatic children with markedly elevated blood lead levels (i.e., starting at 40-60 μg/dl and ranging upwards to 70-80 μg/dl and higher values).

The magnitude of lead's effects on IQ at the high exposure levels evaluated in these studies is difficult to estimate precisely due to variations in measurement instruments used, variations in the extent to which various confounding factors were controlled for in the statistical analyses, and the fact that many of the referent control groups tended to have what are now recognized to be elevated blood lead levels (i.e., averaging in the 20-40 μg/dl range). Focusing on estimates of full-scale IQ deficits, Rummo (1974; Rummo et al., 1979) observed a decrement of approximately 16 IQ points on the McCarthy GCI for postencephalopathic children with blood lead values exceeding 80 μg/dl. Asymptomatic children with long-term lead exposures yielding mean blood lead values of 68 μg/dl experienced an average 5-point IQ (GCI) decrement, whereas short-term lead-exposed subjects with blood lead levels around 60 μg/dl showed no decrement compared to controls. The de la Burde subjects, with blood lead levels averaging 58 μg/dl, had a mean Stanford-Binet IQ decrement of 5 points upon first testing (de la Burde and Choate, 1972) and 3 points upon follow-up testing several years later (de la Burde and Choate, 1975). Ernhart originally reported an average 10 point IQ (GCI) decrement for children with blood lead values in the 40-70 μg/dl range upon first testing (Perino and Ernhart, 1974) and 12 points upon follow-up 5 years later (Ernhart et al., 1981). However, these reported large decrements appear to be due in part to confounding by uncontrolled covariates in the original published data calculations and, upon reanalysis of the data (with better control for confounding variables and with errors corrected), are apparently notably reduced, although the amount of the reduction was not clearly specified in the submitted reanalyses. While it could be argued that the Rummo and de la Burde decrements would also be reduced in size if better control for confounding variables were employed, use of control subjects with lower lead exposures (e.g., <10 μg/dl) could also logically be expected to result in offsetting influences on IQ. Thus, it seems warranted to conclude that the average decrements of about 5 IQ points observed in the de la Burde and Rummo studies represent a reasonable estimate of the magnitude of full-scale IQ decrements associated with notably elevated blood lead levels ($\bar{X} \cong$ 50-70 μg/dl) in asymptomatic children.

12.4.2.2.2.2 General population studies. These studies evaluated samples of non-overtly lead intoxicated children drawn from and thought to be representative of the general pediatric population. They generally aimed to evaluate asymptomatic children with lower lead body burdens than those of high-risk children evaluated in most of the above clinic-type studies.

A pioneering general population study was reported by Needleman et al. (1979), who used shed deciduous teeth to index lead exposure. Teeth were donated from 70 percent of a total population of 3329 first and second grade children from two towns near Boston. Almost all children who donated teeth (2146) were rated by their teachers on an 11-item classroom behavior scale devised by the authors to assess attention disorders. An apparent dose-response function was reported for ratings on the behavior scale, not taking potentially confounding variables into account. After excluding various subjects for control reasons, two groups (<10th and >90th percentiles of non-circumpulpal dentine lead levels) were provisionally selected for further in-depth neuropsychologic testing. Later, some provisionally eligible children were also excluded for various reasons, leaving 100 low-lead (<10 ppm dentine lead) children for comparison with 58 high-lead (>20 ppm dentine lead) children in statistical analyses reported by Needleman et al. (1979). A preliminary analysis on 39 non-lead variables showed significant differences between the low- and high-lead groups for age, maternal IQ and education, maternal age at time of birth, paternal SES, and paternal education. Some of these variables were entered as covariates into an analysis of covariance along with lead. Significant effects ($p < 0.05$) were reported for full-scale WISC-R IQ scores, WISC-R verbal IQ scores, for 9 of 11 classroom behavior scale items, and several experimental measures of perceptual-motor behavior.

Additional papers published by Needleman and coworkers have reported results of the same or further analyses of the data discussed in the initial paper by Needleman et al. (1979). For example, a paper by Needleman (1982) provided a summary overview of findings from the Needleman et al. (1979) study and findings reported by Burchfiel et al. (1980) that are discussed later in Section 12.4.2.2.2.7 concerning EEG patterns for a subset of children included in the 1979 study. Needleman (1982) summarized results of an additional analysis of the 1979 data set reported elsewhere by Needleman et al. (1982). More specifically, cumulative frequency distributions of verbal IQ scores for low- and high-lead subjects from the 1979 study were reported by Needleman et al. (1982), and the key point made was that the average IQ deficit of four points demonstrated by the 1979 study did not just reflect children with already low IQs having their cognitive abilities further impaired. Rather, the entire distribution of IQ scores across all IQ levels was reported to be shifted downward in the high-lead group, with none of the children in that group having verbal IQs over 125. Another paper, by Bellinger and Needleman (1983), provided still further follow-up analyses of the original (Needleman et al., 1979) data set, focusing mainly on comparison of the low- and high-lead children's observed versus expected IQs based on their mother's IQ. Bellinger and Needleman reported that regression analyses showed that IQs of children with elevated levels of dentine lead (>20 ppm) fell below those expected based on their mothers' IQs and that the amount by which a child's IQ fell below the expected value increased with increasing dentine lead levels in a nonlinear fashion. Scatter plots of IQ residuals by dentine lead levels, as illustrated and discussed by Bellinger and Needleman (1983), indicated that regressions for the control children with dentine lead below 10 ppm and for high-lead children with 20-29.9 ppm dentine lead did not reveal significant associations between increasing lead levels in that range and IQ residuals. This is in contrast to statistically significant ($p < 0.05$) correlations found between IQ residuals and dentine lead for high-lead group children with 30-39. ppm dentine lead levels.

The Needleman et al. (1979) study and spin-off analyses published later by Needleman and coworkers were critically evaluated by the same Expert Committee on Neurobehavioral Evaluations noted above that was convened by EPA in March, 1983, to evaluate the Perino and Ernhart (1974) and Ernhart et al. (1981) studies. The Committee's report (Expert Committee, 1983) noted methodological problems with certain of the the published analyses and findings reported by Needleman et al. (1979) or in subsequent papers by Needleman and coworkers concerning additional analyses of the same data set. The Committee also recommended that the Needleman data set be reanalyzed. Reanalyses carried out in response to the Committee's recommendations have been reported by Needleman (1984), Needleman et al. (1985), and U.S. EPA's Office of Policy Analysis (1984) as confirming the published findings on significant associations between elevated dentine lead levels and decrements in IQ, after correcting errors in data calculations detected in earlier published analyses and using alternative model specifications that incorporated better control for potentially confounding factors.

The average magnitude of the full-scale IQ decrement attributable to lead was estimated in the original published Needleman analyses to be about 4 points after control for confounding factors. Based upon the reanalyses submitted, the size of the full-scale lead effect appears to remain about the same (i.e., around 4 points) after controlling for confounding variables. It is, however, extremely difficult to define with confidence quantitative dose-response relationships based on the Needleman data, beyond the statement that average IQ decrements of about 4.0 points appear to be associated with lead exposure levels experienced by the Needleman high-lead group. Among that group, statistically significant ($p < 0.05$) IQ decrements appear to remain (after controlling for confounding variables) for children with 30-39.9 ppm dentine lead levels, but not for children with 20-29.9 ppm or lower dentine lead levels, as reported by Bellinger and Needleman (1983). Only limited data exist by which one might attempt to estimate blood lead values likely associated with the observed IQ effects; and the available information points broadly toward an average blood lead concentration in the 30-50 µg/dl range. An average 4-point full-scale IQ decrement associated with average blood lead values in that range would be consistent with the mean 5-point decrement estimated earlier to occur at somewhat higher average blood lead levels of 50-70 µg/dl.

Recently, Bellinger et al. (1984b) followed up on the academic performance of a subset of the children initially evaluated by Needleman et al. (1979). Of the 118 first and second grade children who were classified into low (<10 ppm) and elevated (≥20 ppm) dentine lead groups by Needleman et al. (1979), 70 were available for study 4 years later. In addition, 71 children with midrange tooth lead levels (10.0-19.9 ppm) were included in the follow-up investigation. Contemporary blood lead levels could not be obtained. Four types of outcome measures were assessed: (1) standardized IQ measures, viz., the most recently available scores for the Otis-Lennon Mental Ability Test, as routinely administered by the school system; (2) teacher ratings, comprising a 24-item pupil-rating scale and the same 11-item scale used by Needleman et al. (1979); (3) indices of school failure, i. e., remedial instruction or grade retention; and (4) direct observation of classroom behavior patterns reflecting inattention, distractibility, etc. Various statistical analyses suggested that only grade retention was clearly associated with past dentine lead levels; other outcomes tended to be in the predicted direction of effect but generally at p values between 0.05 and 0.15. Of some note is the fact that the teacher rating scale revealed no effect of lead, a finding that contrasts with earlier results of Needleman et al. (1979) and a more recent replication (albeit without control for social factors) by Yule et al. (1984).

A study of urban children in Sydney, Australia (McBride et al., 1982) involved 454 preschoolers (aged 4-5 yr) with blood lead levels of 2-29 µg/dl. Children born at the Women's Hospital in Sydney were recruited via personal letter. No blood lead measures were available on non-participants. Blood levels were evaluated at the time of neurobehavioral testing, but earlier exposure history was apparently not assessed. Using a multiple statistical comparison procedure and Bonferroni correction to protect against study-wise error, no statistically significant differences were found between two groups with blood lead levels more than one standard deviation above and below the mean (>19 µg/dl versus <9 µg/dl) on the Peabody Picture Vocabulary IQ Test, on a parent rating scale of hyperactivity devised by Rutter, or on three tests of motor ability (pegboard, standing balance, and finger tapping). In one test of fine motor coordination (tracking), five-year old boys in the higher lead group performed worse than boys in the lower lead group. In one test of gross motor skill (walking balance), results for the two age groups were conflicting. This study suffers from many methodological weaknesses and cannot be regarded as providing evidence for or against an effect of low-level lead exposures in non-overtly lead intoxicated children. For example, a comparison of socioeconomic status (father's occupation and mother's education) of the study sample with the general population showed that it was higher than Bureau of Census statistics for the Australian work force as a whole. Also, there was apparently some self-selection bias due to a high proportion of professionals living near the hospital, and certain other important demographic variables, such as mother's IQ, were not evaluated.

Another recent large-scale study (Smith et al., 1983) of tooth lead, behavior, intelligence, and a variety of other psychological skills was carried out in a general population sample of over 4000 children aged 6-7 years in three London boroughs. Of the 2663 children who donated shed teeth for analysis, 403 children were selected to form six groups, one each of high (8 µg/g or more), intermediate (5-5.5 µg/g), and low (2.5 µg/g or less) tooth lead levels for two socioeconomic groups (manual versus non-manual workers). Parents were intensively interviewed at home regarding parental interest and attitudes toward education and family characteristics and relationships. The early history of the child was then studied in school using tests of intelligence (WISC-R), educational attainment, attention, and other cognitive tasks. Teachers and parents completed the Conners behavior questionnaires. Results showed that intelligence and other psychological measures were strongly related to social factors, especially social grouping. Lead level was linked to a variety of factors in the home, especially the level of cleanliness and, to a lesser extent, maternal smoking. Before correcting for confounding factors, there were significant associations between lead and full-scale IQ scores; however, upon correcting for confounding factors, there were no statistically significant associations between lead level and IQ or academic performance. Also, when rated by teachers (but not by parents), there were small, reasonably consistent (but not statistically significant) tendencies for high-lead children to show more behavioral problems after the different social covariables were taken into account statistically.

The Smith et al. (1983) study has much to recommend it: (1) a well-drawn sample of adequate size; (2) three tooth lead groupings based on well-defined classifications minimizing overlaps of exposure groupings based on whole tooth lead values, including quality-controlled replicate analyses for the same tooth and duplicate analyses across multiple teeth from the same child; (3) blood lead levels on a subset of 92 children which correlated reasonably well with tooth lead levels ($r = 0.45$); (4) cross-stratified design of social groups; (5) extensive information on social covariates and exposure sources; and (6) statistical control for potentially confounding covariates in the analyses of study results. It should also be noted that further statistical analyses of the Smith data, using tooth lead as a continuous variable or finer-grain categorization of subjects into eight tooth lead exposure groups, have recently been reported (Pocock and Ashby, 1985) to confirm no statistically significant associations between tooth lead and IQ across the entire spectrum of lead exposure levels present among the study population. Interestingly, the average full-scale IQ values for the medium- and high-lead groups in the Smith study were 2 points below the average value for the control group. Also, blood lead values for subsets of the children in the medium and high groups averaged 12-15 µg/dl (with all but one <30 µg/dl) upon sampling within a few months of neuropsychologic testing around age six. Somewhat higher blood lead values may have been obtained if sampled at earlier ages for these children (given typical peaking of blood leads seen in preschool children), but likely would have still fallen mainly in the 15-30 µg/dl range.

Harvey et al. (1983, 1984) also recently reported on a study involving 189 children, average age 2.5 years and 15.5 µg/dl blood lead, from the inner city of Birmingham, England. The investigators utilized a wide range of psychometric tests, behavioral measures of activity level, and psychomotor performance. They found that blood lead made no significant contribution to IQ decrements after appropriate allowance had been made for social factors, although, consistent with findings from the Lansdown et al. (1986) study discussed below, a stronger correlation between IQ and blood lead levels was found in children of manual workers ($r = -0.32$) than in children of non-manual workers ($r = +0.06$). Strengths of this study are the following: (1) a well-drawn sample; (2) extensive evaluation of 15 confounding social factors; (3) a wide range of abilities evaluated; and (4) blind evaluations. The finding of no significant associations between lead and IQ decrements at the relatively low blood levels evaluated are consistent with the Smith study results discussed above for children in the same exposure range.

Yule et al. (1981) carried out a pilot study on the effects of low-level lead exposure on 85 percent of a population of 195 children aged 6-12 years, whose blood lead concentrations had been determined some nine months earlier as part of a European Economic Community survey. The blood lead concentrations ranged from 7 to 32 µg/dl, and the children were assigned to four quartiles encompassing the following values: 7-10 µg/dl; 11-12 µg/dl; 13-16 µg/dl; and 17-32 µg/dl. The tests of achievement and intelligence were similar to those used in the Lansdown et al. (1974) and Needleman et al. (1979) studies. Significant associations were reported between blood lead levels and decrements in IQ (full-scale IQ scores averaged ~7 points lower for the highest lead group), as well as lower scores on tests of reading and spelling, but not mathematics (Yule et al., 1981). These differences in performance (although reduced in magnitude) largely remained statistically significant at $p < 0.05$ after age, sex, and father's occupation were taken into account. However, other important potentially confounding social factors such as parental IQ were not controlled in this study, and the investigators cautioned against interpretation of their results as evidence of relationships between lead and IQ or functioning at school without further confirmatory results obtained after better control of social factors and other confounding variables.

Lansdown et al. (1986) replicated their earlier pilot study (Yule et al., 1981) with 194 children ($\bar{X}$ age = 8.8 yr) living in a predominantly working area of London near a busy roadway. In this second, better designed study, a lengthy structured interview yielded data on sources of exposure, medical history, and many potentially confounding variables, including parental IQ and social factors. Analyses of covariance were used to evaluate the effects of lead and other factors on WISC-R verbal, performance, and full-scale IQ scores, as well as reading accuracy and comprehension scores, for children with low (7-12 μg/dl) versus elevated (13-24 μg/dl) blood lead levels. No significant effect of lead was evident even before considering social class. However, there was some suggestion of a trend in effects on IQ in the manual working-class children when compared with non-manual working-class children.

In another study, Yule and Lansdown (1983) evaluated 302 children ($\bar{X}$ age = 9 yr) living in Leeds, England. Tests and procedures similar to those employed in the previous two studies were used and, in addition, a reaction time test was employed (Hunter et al., 1985). The Leeds children were divided, for statistical analyses of the data, by (1) social class (manual versus non-manual) and (2) blood lead level (low = 5-11 μg/dl; high = 12-26 μg/dl). As in the London replication study, no statistically significant relationships for any of the IQ or reading performance scores were found even before social class was controlled for in the statistical analyses. The high-lead children averaged essentially identical or very slightly better than control subjects on several outcomes. On the other hand, small but statistically significant ($p < 0.05$) changes in reaction time (shorter for 3-sec delays; longer for 12-sec delays) were found and appeared to parallel a similar pattern of reaction time effects of larger magnitude reported by Needleman et al. (1979) for American children with higher lead exposures. Analyses of covariance, controlling for age, revealed that the reaction-time differences between low- and high-lead children in Leeds were only significant for the younger children (aged 6-10 yr) but not for the older children (aged 11-14 yr).

Another paper by Yule et al. (1984) reported on the use of three different teacher questionnaires (Needleman, Rutter, and Conners) to assess attention deficits in the same children evaluated in their earlier report (Yule et al., 1981). While there were few differences between groups on the Rutter Scale, the summed scores on the Needleman questionnaire across the blood lead groupings approached significance ($p = 0.096$). Three of the questionnaire items showed a significant dose-response function ("Day Dreamer," "Does not Follow Sequence of Direction," "Low Overall Functioning"). Nine of 11 items were highly correlated with children's IQ. Therefore, the Needleman questionnaire may be tapping IQ-related attention deficits as opposed to measures of conduct disorder and socially maladaptive behavior (Yule et al., 1984). The hyperactivity factors on the Conners and Rutter scales were reported to be related to blood lead levels (7-12 versus 13-32 μg/dl), but the authors noted that caution is necessary in interpreting their findings in view of the crude measures of social factors available and the differences between countries in diagnosing attention deficit disorders. Moreover, since the blood lead values reported were determined only once (nine months before psychological testing), earlier lead exposure may not be fully reflected in the reported blood lead levels. However, even if somewhat higher earlier, it is likely that the blood leads still mainly fell in the 15-30 μg/dl range for the higher two quartile groups.

Two recent reports by Schroeder and his colleagues (Schroeder et al., 1985; Schroeder and Hawk, 1986) are of particular importance to the issue of lead's effects on children's cognitive functioning. Although these studies dealt with children who had been identified through lead-screening programs or who were potentially at risk for elevated lead exposure, the actual blood lead levels measured in these children were, overall, in line with or not much higher than the levels in the general population studies discussed above.

Schroeder et al. (1985) evaluated 104 lower SES children, ages 10 months to 6.5 years. Approximately half of the children (age <30 months) were tested on the Bayley Scales of Mental Development; the remainder of the subjects (age >30 months) were tested on the Stanford-Binet Intelligence Scale. Several other variables were also assessed, including Caldwell and Bradley (1979) HOME scores and parental IQ, SES, education, and employment. Venous blood samples obtained on the day of testing were analyzed for lead concentrations and ranged from 6 to 59 µg/dl ($\bar{X} \cong 30$ µg/dl). Statistical analysis of the data involved a form of hierarchical backward stepwise regression. Lead was found to be a significant ($p < 0.01$) source of the effect on IQ scores in these children after controlling for SES, HOME score, maternal IQ, and other social factors. SES was the only other variable to reach statistical significance ($p < 0.001$); other variables apparently failed to reach significance because of collinearity with SES. A corollary study of the same children by Milar et al. (1981a) found no association between lead exposure and hyperactivity.

Fifty of the children were re-examined 5 years later, at which time all blood lead levels were 30 µg/dl or lower. In addition to re-evaluating the children with the Stanford-Binet IQ test, the investigators repeated SES and maternal IQ (but not HOME) measurements. Although the 5-year follow-up IQ scores were negatively correlated with both contemporary and initial blood lead levels, the effect of lead was not significant after covariates (especially SES) were included in the regression model. It is interesting to note also that the correlation between maternal and child IQ was only about 0.06 for children with initial blood lead levels of 31-56 µg/dl, but returned to a nearly normal value of 0.45 after 5 years, when blood lead levels had dropped. Similar findings have been reported by Perino and Ernhart (1974) and Bellinger and Needleman (1983), and have been used to argue that an environmental factor (i.e., lead) disrupts the normal mother-child IQ correlation of about 0.50. Thus, Schroeder et al.'s (1985) finding provides further, indirect evidence of lead's disruptive effect on children's cognitive functioning at blood lead levels in the range of approximately 30-60 µg/dl.

Schroeder and Hawk (1986) replicated the above study with 75 Black children, all of low SES and ranging in age from 3 to 7 years. Blood lead levels averaged 21 µg/dl (range: 6-47 µg/dl). Backward stepwise multivariate regression analysis revealed a highly significant relationship between contemporary blood lead level and IQ ($p < 0.0008$); the effect was nearly as striking (p <0.002) whether maximum or mean blood lead values (from health department records) were used. No other covariate achieved significance at p = 0.05 in this analysis, although maternal IQ was closest. SES was not a significant covariate in this study because SES was uniformly low. (Further analyses showed HOME scores to be significantly correlated with blood lead levels and to be collinear with maternal IQ and SES.) As Figure 12-2 indicates, the effect of blood lead level on IQ appeared to extend linearly across the entire range of blood lead concentrations. In fact, 78 percent of the subjects had blood lead levels below 30 µg/dl.

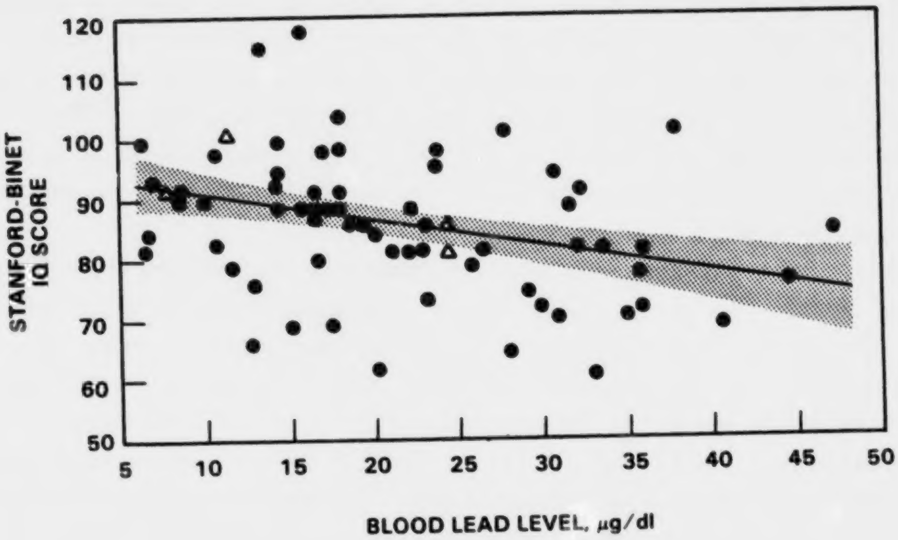


Figure 12-2. Regression of IQ scores against blood lead levels, with 95% confidence band. Double values indicated by triangle.

Source: Schroeder and Hawk (1986).

Another recent investigation that should be noted here has been reported by Silva et al. (1986b). This preliminary study investigated cognitive development and behavior problems in

579 11-year-old children in Dunedin, New Zealand. Higher SES groups were significantly overrepresented in this sample, but the correlation between blood levels and SES was near zero. The mean blood lead level at age 11 was 11.1 µg/dl (SD = 4.91). No significant effects on IQ were evident from an analysis of WISC-R scores. Regression analyses and multiple correlations were performed on scores from a reading ability test, the Rutter parent and teacher questionnaires, and other assessments of children's inattention and hyperactivity derived from parent and teacher reports. The contribution of blood lead levels to the explained variance for the reading ability scores was nonsignificant. However, five of the six remaining assessments of children's behavior showed significant increases in the amount of explained variance when the blood lead variable was added. Although blood lead accounted for only 0.8-1.2 percent of the additional variance, the results nonetheless indicate some association between lead exposure and small but significant adverse effects on behavior in older children, even after allowance for certain background factors (e.g., maternal verbal ability, maternal depression, a composite index of social disadvantage). A complementary report by Silva et al. (1986a) noted that some of the children in the Dunedin pilot study had had significant exposure to lead through paint-stripping activities in the home. Although only two subjects had blood lead levels above 30 µg/dl at the time of testing, this backgorund information points up the need for earlier and more precise characterization of long-term lead exposure for an accurate interpretation of the Dunedin findings.

None of the general population studies reviewed here individually provide definitive evidence for or against neuropsychologic deficits being associated with relatively low body lead burdens in non-overtly lead-intoxicated children representative of general pediatric populations. The recent report by Schroeder and Hawk (1986) indicates a highly significant linear relationship between a measure of IQ and blood lead levels over the range of 6 to 47 µg/dl. This effect was almost equally as strong regardless of whether contemporary, past maximum, or mean blood lead levels were used in the analysis. Because the subjects were all Black children of uniformly low socioeconomic status, SES was not a significant covariate in the analysis. On the other hand, this feature of the study limits the applicability of the findings to the general U.S. population of children. It is possible that SES and lead exposure interact such that IQ is affected by blood lead at lower SES levels but not at higher SES levels (cf. Schroeder et al., 1985). Findings of stronger correlations between IQ and blood lead levels in children of manual working class fathers (Harvey et al., 1983, 1984; Yule and Lansdown, 1983; Lansdown et al., 1986) are consistent with this supposition (cf. Winneke and Kraemer, 1984). If true, this interactive relationship would suggest that lower socioeconomic status places children at greater risk to the deleterious effects of low-level lead exposure on cognitive ability. However, as results from Schroeder et al. (1985) and Schroeder and Hawk

(1986) indicate, other variables such as HOME scores and maternal IQ may covary with SES. Other work (e.g., Milar et al., 1980; Dietrich et al., 1985b) points to the home environment as a significant predictor of lead exposure. This close relationship between SES, quality of home environment, and lead exposure suggests that SES may not be the sole determiner of increased risk for cognitive impariment. Further research is needed to disentangle the relative contributions of these variables to the neurotoxic effects of lead.

Of the other studies reviewed here, the Needleman analyses may be interpreted as providing acceptable evidence for full-scale IQ deficits of about 4 points and other neurobehavioral deficits being associated with lead exposures of American children resulting in dentine lead values that exceed 20-30 ppm and likely average blood lead values in the 30-50 µg/dl range. The report of recent analyses by Schroeder et al. (1985) supports this conclusion, even after the major influence of SES was allowed for in the analyses. However, their findings indicate that the effect of blood lead on IQ could not be detected five years after the original assessment. A follow-up by Bellinger et al. (1984b) of the children studied by Needleman et al. (1979) suggests that other measures of classroom performance may show long-term effects of early lead exposure more effectively than IQ measures (see also Silva et al., 1986b). Shaheen (1984) has also questioned the sensitivity of IQ scores and has suggested that the variability in outcomes of studies of lead's effects on neuropsychological functioning in children may originate with differences in the ages at which children are subjected to toxic lead exposures.

For the most part, the remaining general population studies reviewed in this section report a lack (with a few exceptions) of statistically significant effects on IQ or other neuropsychologic measures. Most of the remaining studies found slightly lower IQ scores for higher-lead exposure groups than for low-lead control groups before correcting for confounding variables, but the differences were typically reduced to 1-2 IQ points and were non-significant (usually even at $p < 0.10$) upon correction for confounding factors. The following conclusions may be stated about these latter results: (1) they are suggestive of relatively minimal (if any) effects of lead on IQ in general populations, especially in comparison to the much larger effects of other factors (e.g., social variables), at the exposure levels evaluated in these studies (blood lead values mainly in the 15-30 µg/dl range); and (2) they are not incompatible with findings of significant lead effects on IQ at higher average blood lead levels (≧30 µg/dl).

The few exceptions to the general pattern noted above warrant comment here. The pilot study by Yule et al. (1981, 1984), which found significant (6-7 point) IQ decrements and poorer ratings on several categories of classroom behavior, has certain methodological limitations; specifically, the study provided only relatively crude control for socioeconomic factors (as noted by the authors) and it failed to take parental IQ into account at all. In comparison to other studies, the reported IQ decrements of about 6-7 points are consistent with neither (1) the maximum 1-2 point IQ differences seen in other general population studies of children with comparable lead exposures (mainly in the 15-30 µg/dl blood lead range), nor (2) the results of clinic studies showing 4-5 point IQ decrements at distinctly higher lead levels (i.e., at >30 µg/dl). However, the findings of altered reaction time patterns by Hunter et al. (1985), which parallel those reported by Needleman at higher exposure levels, are somewhat more credible and appear to argue for probable effects of lead on attention or vigilance functions at levels extending below 30 µg/dl and, possibly, down to as low as 15-20 µg/dl.

12.4.2.2.2.3 Smelter area studies. The smelter studies evaluated children with elevated lead exposures associated with residence in cities or elsewhere in close proximity to lead-emitting smelters. Most of the early studies, conducted in the 1970s, found mixed results even though evaluating children with blood lead levels typically in excess of 30 µg/dl. Because of methodological weaknesses, however, virtually all of the early studies must be viewed as inconclusive.

For example, in an early study of this type Lansdown et al. (1974) reported a relationship between blood lead level in children and the distance they lived from lead-processing facilities, but no relationship between blood lead level and mental functioning. However, only a minority of the lead-exposed cohort had blood lead levels markedly differing from control subjects with elevated blood lead levels (<40 µg/dl). Furthermore, this study failed to adequately consider important confounding factors such as socioeconomic status.

In another study, Landrigan et al. (1975) found that lead-exposed children living near an El Paso, Texas, smelter scored significantly lower than matched controls on measures of performance IQ and finger-wrist tapping. The control children in this study were, however, not well matched by age or sex to the lead-exposed group, although the results remained statistically significant after adjustments were made for age differences. In contrast, McNeil and Ptasnik (1975) found little evidence of lead-associated decrements in cognitive abilities in another sample of children living near the same lead smelter in El Paso. These children who were generally comparable medically and psychologically to matched controls living elsewhere in the same city except for the direct effects of lead (blood lead level, free erythrocyte protoporphyrin levels, and X-ray findings). An extensive critique of these two El Paso studies was performed by another expert committee (see Muir, 1975), which concluded that no reliable conclusions could be drawn from either of the published studies in view of various methodological and other problems affecting their conduct and statistical analyses.

A later study by Ratcliffe (1977) of children living near a battery factory in Manchester, England, found no significant associations between blood lead levels sampled at two years of age (28 μg/dl versus 44 μg/dl in low- versus high-lead groups) and testing done at age five on the Griffiths Mental Development Scales, the Frostig Developmental Test of Visual Perception, a pegboard test, or a behavioral questionnaire. The differences in scores, although small, were somewhat better for the low-lead exposure children than for the higher exposure group. The small sample size (23 low-lead and 24 high-lead children), inadequate control for parental IQ, and the failure to repeat blood lead assays at age five weaken this study. Variations in blood lead levels occurring after age two among control children may have lessened exposure differences between the low- and high-lead groups, and larger sample sizes would have better allowed for detection of any lead effects present.

The more recent smelter studies, described next, provide assessments that generally accord somewhat greater attention to control for potentially confounding factors. Also, some of the studies assessed larger samples of children, presumably allowing more accurate estimation of any lead effects present.

Two studies by Winneke and colleagues, the first a pilot study (Winneke et al., 1982a) and the second an extended study (Winneke et al., 1983), employed tooth lead analyses analogous to some of the studies already discussed above. In the pilot study, incisor teeth were donated by 458 children aged 7-10 years in Duisburg, Germany, an industrial city with airborne lead concentrations between 1.5 and 2.0 $\mu g/m^3$. Two extreme exposure groups were formed, a low-lead group with 2.4 μg/g mean tooth lead level (n = 26) and another, high-lead group with 7 μg/g mean tooth lead level (n = 16). These groups were matched for age, sex, and father's occupational status. The two groups did not differ significantly on confounding covariates, except that the high-lead group showed more perinatal risk factors. Parental IQ and quality of the home environment were not among the 52 covariables examined. The authors found a marginally significant decrease ($p < 0.10$) of 5-7 IQ points and a significant decrease in perceptual-motor integration ($p < 0.05$), but no significant differences in hyperactivity as measured by the Conners Teachers' Questionnaire administered during testing. As with the Yule et al. (1981) study, the inadequacy of statistical or other control for background social variables and parental IQ (as well as group differences in perinatal factors) weaken this study; the investigators cautioned against interpretation of their results as evidence for low-level lead exposure effects in the absence of further, confirmatory results from larger, better controlled studies (such as those conducted by them elsewhere as described below).

In their second study, Winneke et al. (1983) evaluated 115 children ($\bar{X}$ age = 9.4 years) living in the lead smelter town of Stolberg, Germany. Tooth lead ($\bar{X}$ = 6.16 ppm, range = 2.0-38.5 ppm) and blood lead levels ($\bar{X}$ = 13.4 μg/dl; range = 6.8-33.8 μg/dl) were significantly correlated ($r = 0.47$; $p < 0.001$) for the children studied. Using stepwise multiple regression analysis, the authors found significant ($p < 0.05$) or marginally significant ($p < 0.10$) associations between tooth lead levels and measures of perceptual-motor integration, reaction-time performance, and four behavioral rating dimensions, including distractibility. This was true even after taking into account age, sex, duration of labor at birth, and socio-heredity background as covariates. However, the proportion of explained variance due to lead never exceeded 6 percent for any of these outcomes, and no significant association was found between tooth lead and WISC verbal IQ after the effects of socio-hereditary background were eliminated.

A third study by Winneke et al. (1984) evaluated neuropsychologic functioning and neurophysiological parameters for 122 children (aged 6-7 yr) living in the Nordenham, FRG area. Performance on a variety of neuropsychologic tests (shortened form of the Hamburg-Wechsler IQ test; reaction-behavior and reaction-time tests, etc.) was evaluated in relation to both concurrently sampled blood lead values ($\bar{X}$ = 8 µg/dl; max. = 23 µg/dl) and umbilical cord blood lead levels (max. = 31 µg/dl). A variety of potentially confounding factors (such as socio-hereditary variables, pre- and postnatal risk factors, etc.) were also assessed and taken into account in a series of stepwise multiple regression analyses in which the effects of confounding factors were successively eliminated and the effects of lead then checked for significance. No significant associations (at $p < 0.05$) were found between either umbilical cord or current blood lead levels and verbal, performance, or total IQ scores estimated from the Hamburg-Wechsler subtests (only the correlation for performance IQ with current blood lead level reached $p < 0.10$). On the other hand, much larger and highly significant correlations were found between socio-hereditary factors and all three types of IQ scores. The investigators remarked on the heavy dependence of the IQ measurements on the social environment and noted that, as in their prior large-scale study (Winneke et al., 1983), it was not possible to convincingly show a lead-dependent decrease in intelligence. Nor were any lead effects found on the Goettinger shape reproduction test of psychomotor performance or for various reaction-time measures. Only in the case of reaction behavior, as indexed by increased errors on the Wiener (Vienna) serial stimulus reaction test, were significant deficits in neuropsychological functioning detected at the low exposure levels (<25-30 µg/dl) evaluated in this study. Certain statistically significant effects on electrophysiological measures of neurophysiological functioning were also observed (as described below in Section 12.4.2.2.2.7).

The above smelter area studies generally do not provide much evidence for cognitive or behavioral deficits being associated with lead exposure in non-overtly lead exposed children, except perphaps for the reaction-behavior deficits reported by Winneke et al. (1984). The lack of convincing evidence for IQ deficits at the blood lead levels (generally 15-30 µg/dl) typifying the pediatric populations studied by Winneke comport well with the same type of findings reported by British investigators (Yule; Smith; Harvey) for general population groups with similar lead exposure ranges. At the same time, the possibility of small neuropsychologic deficits being associated with lead exposure in apparently asymptomatic children at the exposure levels studied cannot be completely ruled out, given the overall pattern of results obtained with the cross sectional study designs employed by Winneke and the British investigators. Small, 1-2 point differences in IQ seen in some of their studies between control and lead exposure groups might in fact be due to lead effects masked by much larger effects of socioeconomic factors, home environment, or parental IQ. At the same time, the very small or nil differences in IQ seen in these studies for children with blood lead levels mainly in the 15-30 µg/dl range suggest that, if the IQ decrements are in fact due to lead, then it is extremely unlikely that any IQ effects (of presumably even smaller magnitude) would be convincingly detectable at lower blood lead levels.

12.4.2.2.2.4 Studies of neuropsychiatrically disordered children. Rather than starting with a known lead-exposed population and attempting to discover evidence of neurobehavioral dysfunction, a number of studies have first identified a population with some recognized disorder and then looked for evidence of elevated lead exposure. For example, a series of studies by David et al. (1972; 1976a,b; 1977; 1979a,b; 1982a,b; 1983; 1985) measured lead levels in diagnosed hyperkinetic children and showed an association between hyperactivity and elevated lead levels. However, whether a disorder such as hyperactivity is the effect or the cause of elevated lead exposure is a difficult issue to resolve. It is possible, for example, that hyperactive children might ingest more lead than normal children because of a greater incidence of pica or even because they stir up more dust-borne lead by their activity. However, David et al. (1977) reported that blood lead levels of hyperactive children with a probable etiology of an organic nature were lower than those of children with no apparent cause (other than lead). This finding suggests that hyperactivity does not necessarily result in elevated lead exposure, but it does not rule out the possibility of a third factor causing both hyperactivity and elevated blood lead levels (see discussion of Gittelman and Eskenazi, 1983, below). Also, a problem common to the studies in question is the lack of adequate information on the children's past exposure to lead, particularly during preschool years when children tend to be at greatest risk to higher exposure levels. As David et al. (1976a) have acknowledged, it is difficult to establish an etiological relationship between lead and behavioral disorders on the basis of retrospective estimations of lead exposure.

A recent study by David et al. (1983) appeared to obviate some of the problems of the correlational approach by experimentally manipulating body lead levels, i.e., by reducing blood lead concentrations through the administration of a chelating agent, penicillamine. The objective was to determine if decreases in body lead would be accompanied by improvements in children's hyperactive behavior, and in short, this was essentially the conclusion drawn by David and his colleagues. In addition, the study compared the effect of the chelating agent with a therapeutic drug of known efficacy, methylphenidate, and found the two treatments to be roughly equivalent in reducing symptoms of hyperactivity.

Although this study by David et al. (1983) was in many respects well designed and executed, certain problems nevertheless cloud its interpretation. As noted by Needleman and Bellinger (1984), the number of subjects per treatment group was rather limited (maximum of 31) and quite unbalanced due in part to a high and disproportionate subject attrition rate. Subjects were particularly prone to drop out of the placebo group, and this imbalance was exacerbated by a "chance preponderance" of subjects assigned to the penicillamine treatment and by later reassignment of some placebo and methylphenidate subjects to the penicillamine group. Questions might also be raised concerning the appropriateness of the statistical treatment of data by David et al. (1983). For example, multivariate analysis of variance (MANOVA) would seem to be more appropriate than separate ANOVAs and multiple t-tests applied to the various outcome measures used to assess the children's behavior. Use of MANOVA would also have helped alleviate the problem of regression toward the mean, which in this case may have created the false impression that "improvements" in behavior, i.e., changes toward more normal behavior, were due to an effect of the treatment. Rutter (1983, p. 313) has also noted that David's multiple group comparisons are not as convincing as an analysis that would utilize individual blood lead and behavior scores (presumably, multivariate regression analysis). Finally, as David et al. (1983) themselves point out, it is clear that lead could be only one of several etiological factors in the causation of hyperkinesis or attention deficit disorders in children and that, at best, their findings pertain only to recognized hyperactive children, not to the general population.

An attempt by Gittelman and Eskenazi (1983) to replicate earlier work by David et al. (1972; 1977) was only partly supportive of the latter's findings. A large group of hyperactive children (n = 103) showed a trend ($p = 0.06$) toward higher chelated lead levels in their urine, but a clear-cut ($p = 0.02$) elevation in lead levels was evident only in paired comparisons with 33 nonhyperkinetic siblings. As Gittelman and Eskenazi (1983) noted, this finding raises the question of why the hyperactive children had higher lead levels than their siblings, given that they shared the same water, air, and home environment. The possibility of a third factor, e. g., a metabolic difference that might affect the ability to excrete lead as well as the occurrence of hyperactivity, cannot be dismissed.

A study of 98 Swedish children with various minor neuropsychiatric disorders (e.g., perceptual-motor dysfunctions, speech disorders, attention deficit problems) found no correlation between the children's disorders and their tooth lead levels (Gillberg et al., 1982). However, comparing the 10 highest and 10 lowest lead-burdened children did reveal a significant difference in a clinical measure of their mean reaction times.

Youroukos et al. (1978) compared the blood lead as well as ALA-D values of 60 Greek children with mental retardation of unknown etiology against 30 mentally retarded children with a known etiology and 30 normal children. The average values of the mentally retarded patients were significantly different from both of the control groups in two regards: blood lead level was higher (30 μg/dl versus 21 μg/dl in both control groups) and, in 14 patients with elevated (≥ 40 μg/dl) blood lead levels, ALA-D activity was significantly lower. Although pica was noted to be common in both groups of mentally retarded children, no child in the study was known to have ever been lead-poisoned.

Work in Scotland has provided information tending to link prenatal lead exposures to the later development of mental retardation. Beattie et al. (1975) identified 77 retarded children and 77 normal children matched on age, sex, and geography. The residence during the gestation of the subject was determined, and a first-flush morning sample of tap water was obtained from the residence. Of 64 matched pairs, no normal children were found to come from homes served with water containing high lead levels (>800 μg/liter), whereas 11 of the 64 retarded children came from homes served with such high-lead water. The authors concluded that pregnancy in a home with high lead in the water supply increases by a factor of 1.7 the risk of bearing a retarded child. In follow-up work, Moore et al. (1977) obtained lead values from blood samples drawn during the second week of life from children studied by Beattie et al. The samples had been obtained as part of routine screening for phenylketonuria and kept stored on filter paper. Blood samples were available for 41 of the retarded and 36 of the normal children in the original study by Beattie et al. Blood lead concentrations in the retarded children were significantly higher than values measured in normal children: the mean for retardates was 1.23 ± 0.43 μmol/liter (25.5 ± 8.9 μg/dl) and for normals was 1.0 ± 0.38 μmol/liter (20.9 ± 7.9 μg/dl). The difference in lead concentrations was significant ($p = 0.02$) by the Mann-Whitney test.

These latter two studies suggest that lead exposure to the fetus during the critical period of brain development may cause perturbations in brain organization that are expressed later in mental retardation syndromes, and they raise for careful scrutiny the issue of postnatal risks associated with intrauterine exposure to lead. Long-term prospective studies of the type described next are beginning to produce results which address that issue.

12.4.2.2.2.5 Prospective Studies of Neurobehavioral Effects of In-Utero or Early Postnatal Lead Exposures. During recent years a number of prospective studies have been initiated in the United States and abroad (Europe, Australia, etc.). These studies emphasize the following: (1) the documentation of lead exposure histories during pregnancy, at birth, and/or postnatally well into later years of childhood; and (2) the evaluation of relationships between such lead exposures and delays in early postnatal physical or neurological development and, also, subsequent alterations in normal neuropsychological and neurophysiological functions. Progress in a number of these studies was discussed at the Second International Conference on Prospective Lead Studies held in April, 1984 (Bornschein and Rabinowitz, 1985).

Initial results have been obtained from two of these studies and are of particular interest here.

As part of a longitudinal study of early developmental effects of lead, Bellinger et al. (1984a) administered Bayley Scales of Infant Development at age 6 months to infants born at a Boston hospital. The infants were classified into three groups according to umbilical cord blood lead levels obtained at birth: low ($\bar{X}$ = 1.8 µ/dl); middle ($\bar{X}$ = 6.5 µg/dl); and high ($\bar{X}$ = 14.6 µg/dl; none exceeded 30 µg/dl). Multiple regression analyses indicated that the "high" cord blood-lead levels were significantly associated with lower covariance-adjusted scores on the Bayley Mental Development Index, but scores on the Psychomotor Development Index were not related to cord blood-lead levels. Infant blood-lead levels sampled at 6 months of age were not associated with scores on either the Mental or Psychomotor Development Index. These data were interpreted by Bellinger et al. (1984a, 1985) as being compatible with the hypothesis that low levels of lead delivered transplacentally to the fetus are toxic to the newborn infant. However, although the results suggest that *in utero* exposure may result in delays in early development during the first 6 months postnatally, the results do not allow estimation of the persistence of the observed delays in postnatal neurobehavioral development.

Dietrich et al. (1985a) also recently reported initial results emerging from a long-term prospective study of infants born in Cincinnati, Ohio. The Bayley Mental Development Index (MDI), Psychomotor Development Scale (PDS), and Infant Behavior Record (IBR) were administered at 3, 6, 12, and 24 months to infants not born at significant biological risk due to non-lead factors (such as low birth weights, etc.). The Home Observation for Measurement of the Environment (HOME) scales (Caldwell and Bradley, 1979) were used to assess and statistically control for relevant factors in the rearing environments of the infants, and blood samples were obtained at birth (umbilical cord blood), 10 days, and every three months thereafter. Geometric mean blood lead levels increased from 6.11 µg/dl at 3 months to 14.87 µg/dl at 12 months and included maximum values of 28, 33, 55, and 46 µg/dl at the 3, 6, 9 and 12 month sampling points. Based on regression analyses between blood lead values at those time points and MDI scores at 3, 6, and 12 months, only an unadjusted negative lag correlation between blood levels at 3 months and MDI at 6 months was significant; but that correlation was substantially reduced and no longer significant after adjustment for HOME scores. Free erythrocyte protoporphyrin levels at 6 months were significantly correlated to 6-month MDI scores and remained so after correction for HOME scores. As for IBR data, only "Sensory Interest" at 12 months was significantly negatively correlated with 6 or 12 month blood lead levels (at $p < 0.05$ and $p < 0.01$, respectively). The lag correlation between 6 month blood leads and 12 month IBR "Sensory Interest" was not significant after adjustment for HOME scores, but the correlation with 12 month blood leads remained significant after adjustment for HOME scores.

The investigators concluded, based on these initial results, that low to moderate lead exposure during the first year of life has only a small impact (if any) on early sensorimotor development.

(More recent significant results from these other longitudinal studies are reviewed and assessed in the Addendum to this document.)

12.4.2.2.2.6 Studies of association of neuropsychologic effects and hair lead levels. Several studies have reported significant associations between hair lead levels and behavioral or cognitive testing endpoints (Pihl and Parkes, 1977; Hole et al., 1979; Hansen et al., 1980; Capel et al., 1981; Ely et al., 1981; Thatcher et al., 1982; Marlowe et al., 1982, 1983, 1985; Marlowe and Errera, 1982). Measures of hair lead are easily contaminated by external exposure and are generally questionable in terms of accurately reflecting internal body burdens (see Chapter 9). Such data, therefore, cannot be credibly used to evaluate relationships between absorbed lead and nervous system effects and are not discussed further here.

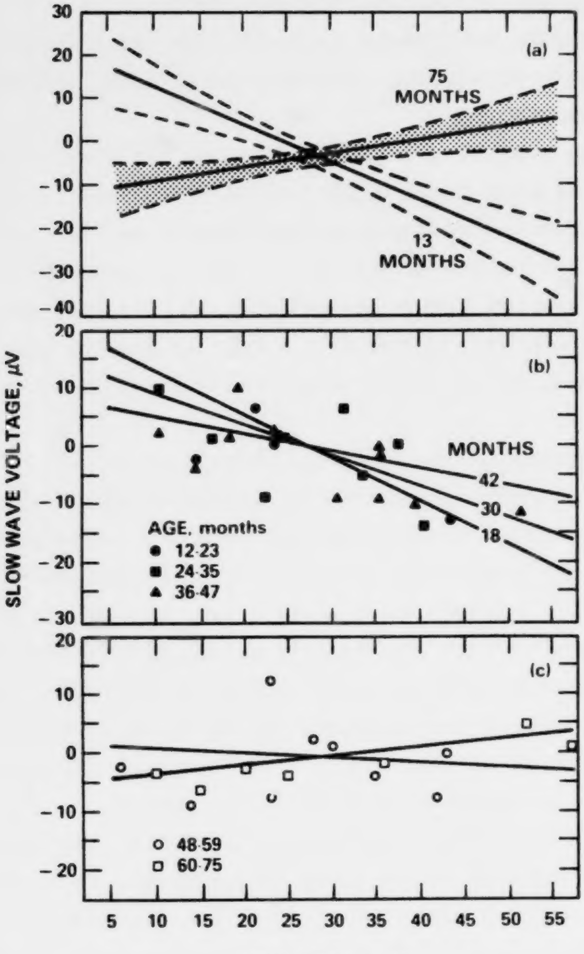
12.4.2.2.2.7 Electrophysiological studies of lead effects in children. In addition to psychometric and behavioral approaches, electrophysiological studies of lead neurotoxicity in non-overtly lead-intoxicated children have been conducted. One such study (Thatcher et al., 1984) reported significant effects on various measures of auditory and visual evoked potentials in lead-exposed children, but the only measure of lead exposure was hair lead, which, as previously noted, is not a suitable index of lead exposure.

Burchfiel et al. (1980) used computer-assisted spectral analysis of a standard EEG examination on 41 children from the Needleman et al. (1979) study and reported significant EEG spectrum differences in percentages of alpha and low-frequency delta activity in spontaneous EEGs of the high-lead children. Percentages of alpha and delta frequency EEG activity and results for several psychometric and behavioral testing variables (e.g., WISC-R full-scale IQ and verbal IQ, reaction time under varying delay, etc.) for the same children were then employed as input variables (or "features") in direct and stepwise discriminant analyses. The separation determined by these analyses for combined psychological and EEG variables ($p < 0.005$) was reported to be strikingly better than the separation of low-lead from high-lead children using either psychological ($p < 0.041$) or EEG ($p < 0.079$) variables alone. Unfortunately, no dentine lead or blood lead values were reported for the specific children from the Needleman et al. (1979) study who underwent the EEG evaluations reported by Burchfiel et al. (1980). Lead-exposure levels associated with the observed EEG effects would appear likely to fall within the same broad 30-50 µg/dl blood lead range estimated earlier for the Needleman IQ deficit observations.

Guerit et al. (1981) examined 79 11-year-old children attending three different schools in the vicinity of a lead smelter and presenting blood lead levels up to 44 µg/dl (averaging less than 30 µg/dl). Children from two distant urban and rural schools served as controls. A neurophysiological function score for each child was based on measures of EEGs, visual evoked potentials, brainstem auditory evoked potentials, and eye movements. Neurophysiological scores were negatively correlated ($p < 0.05$ by Spearman rank correlation coefficient) with blood lead and FEP levels for the children from one of the smelter area schools, but the authors attributed this finding to the inclusion of four children who were left-handed or suffering from external ear pathology. Chi-square tests of neurophysiological scores as a function of blood lead or FEP groupings based on the total study population were all non-significant. Note that comparatively low power nonparametric statistical tests were employed in this study because of the qualitative or ordinal nature of the data. However, the use of more detailed quantitative measures of neurophysiological function would have enabled the investigators to employ more powerful parametric statistics, with possibly different outcomes from their analyses.

The relationship between low-level lead exposure and neurobehavioral function (including electrophysiological responses) in children aged 13-75 months was extensively explored in another study, conducted at the University of North Carolina in collaboration with the U.S. Environmental Protection Agency. Psychometric evaluation revealed a significant lead-related IQ decrement at the time of initial evaluation (Schroeder et al., 1985), as noted previously. No relationship between blood lead and hyperactive behavior (as indexed by standardized playroom measures and parent-teacher rating scales) was observed in these children (Milar et al., 1981a). On the other hand, electrophysiological assessments, including analyses of slow cortical potentials during sensory conditioning (Otto et al., 1981) and EEG spectra (Benignus et al., 1981), did provide evidence of CNS effects of lead in the same children. A significant linear relationship between blood lead (ranging from 6 to 59 µg/dl) and slow wave voltage during conditioning trials was observed (Otto et al., 1981), as depicted in Figure 12-3. Analyses of quadratic and cubic trends, moreover, did not reveal any evidence of a threshold for this effect. The slope of the blood lead x slow wave voltage function, however, varied systematically with age. No effect of blood lead on EEG power spectra or coherence measures was observed, but the relative amplitude of synchronized EEG between left and right hemispheres (gain spectra) increased relative to blood lead levels (Benignus et al., 1981). A significant cubic trend for gain between the left and right parietal lobes was found with a major inflection point at 15 µg/dl. This finding suggests that EEG gain is altered at blood lead levels as low as 15 µg/dl, although the clinical and functional significance of this measure has not been established.

A follow-up study of slow cortical potentials and EEG spectra in a subset (28 children aged 35-93 months) of the original sample was carried out two years later (Otto et al., 1982).



PbB LEVEL, μg/dl

Figure 12-3. (a) Predicted slow wave voltage and 95% confidence bounds in 13- and 75-month-old children as a function of blood lead level. (b) Scatter plots of slow wave data from children aged 13 to 47 months with predicted regression lines for ages 18, 30, and 42 months. (c) Scatter plots for children aged 48 to 75 months with predicted regression lines for ages 54 and 66 months. These graphs depict the linear interaction of blood lead and age.

Source: Otto et al. (1981).

Slow wave voltage during sensory conditioning again varied as a linear function of blood lead, even though the mean lead level had declined by 11 µg/dl (from 32.5 µg/dl to 21.1 µg/dl). Although the EEG gain effect did not persist, the similarity of slow wave voltage results obtained at initial and follow-up assessments suggests that the observed alterations in this parameter of CNS function were persistent, despite a significant decrease in the mean blood lead level during the two-year interval.

In a five-year follow-up study on a subset of the same children, Otto et al. (1985) found that slow wave voltage varied as a function of current blood lead level during active conditioning, but not during the passive conditioning test used in earlier studies. In the passive test, a tone was paired with a short blackout of a silent cartoon. The active test was similar except that children pressed a button to terminate the blackout and resume the cartoon. Although the brain response elicited by the active test is greater than that produced by the passive test, the active test cannot be performed reliably by children under five years of age.

In addition to the experimental conditioning tests, Otto et al. (1985) used two clinically validated measures of sensory function, the pattern-reversal visual evoked potential (PREP) and the brainstem auditory evoked potential (BAEP). Exploratory analysis of PREPs revealed increased amplitude and decreased latency of certain components as a linear function of original blood lead levels. Although these results were contrary to predictions, the findings are consistent with the results of Winneke et al. (1984), who found an association between increased blood lead level and decreased latency in the primary positive component of PREPs in children. BAEP results of the five-year follow-up study also indicated significant associations between original blood lead levels and increased latencies of two components (waves III and V), indicative of auditory nerve conduction slowing.

Otto and his coworkers (Otto, 1986; Robinson et al., 1985) recently reported the results of a replication study with an independent group of children 3 to 7 years old. Blood lead levels ranged from 6 to 47 µg/dl at the time of testing. Psychometric data from this study (Schroeder and Hawk, 1986) have been reviewed above. Sensory conditioning was limited to the passive test due to the age range of the children. Contrary to earlier findings (Otto et al., 1981, 1982), slow wave voltage did not vary with blood lead levels. Differences between the two groups studied, however, may have contributed to the discordant results. Children in the earlier studies were somewhat younger (1-6 versus 3-7 years) and were exposed by different routes (secondary occupational exposure versus lead paint and contaminated soil) than children in the replication study (see review by Otto, 1986). Until further studies are undertaken to clarify the inconsistent slow wave results, earlier findings must be interpreted cautiously.

Inconsistencies in PREP and BAEP results between the five-year follow-up and replication studies were also found. Only one PREP amplitude measure varied systematically with blood lead levels in the replication study, and this was in the opposite direction from previous findings. BAEP results of the replication study were considerably more complex and only partially consistent with the five-year follow-up study. Several BAEP latency measures showed a curvilinear relationship to maximal blood lead levels, whereas a simple linear relationship was observed in the earlier study. That is, BAEP latencies in the replication study decreased as blood lead levels rose from 6 to 25 µg/dl, but increased at higher PbB levels. The descending limb of this curve paralleled the findings of Winneke et al. (1984), who observed faster peripheral nerve conduction velocities as well as decreasing latency in the primary positive PREP component of children with blood lead levels up to 23 µg/dl. On the other hand, the ascending limb of the BAEP latency curve was consistent with the five-year follow-up results. Moreover, the I-V interpeak latency, a measure of central transmission time in the auditory pathway, increased linearly with increasing blood lead levels in the replication study. In addition, hearing threshold, a reflection of peripheral auditory system function, increased directly with lead levels. Although hearing threshold did not vary with blood lead level in the five-year follow-up study (Otto et al., 1985), this finding bears further investigation in view of other reports suggesting impaired auditory processing in lead-exposed children (de la Burde and Choate, 1975; Needleman et al., 1979).

In summary, these electrophysiological studies provide suggestive evidence of lead-related effects on CNS function in children at blood lead levels considerably below 30 µg/dl, but inconsistent findings across studies require clarification. Linear dose-response relations have been observed in slow-wave voltage during conditioning (Otto et al., 1981, 1982, 1985), BAEP latency (Otto et al., 1985), PREP latency (Otto et al., 1985; Winneke et al., 1984), and PREP amplitude (Otto, 1986; Otto et al., 1985a), although the specific components affected and direction of effect varied across studies. Sensory evoked potentials, in particular, hold considerable promise as sensitive, clinically valid nervous system measures unaffected by social factors that tend to confound traditional psychometric measures (Halliday and McDonald, 1981; Prasher et al., 1981). BAEPs, for instance, are not altered by changes in attention or level of consciousness. Reliable BAEPs can be recorded in (sedated) children between the ages of one and five, the most vulnerable period for lead poisoning as well as the most difficult period for most types of neurobehavioral testing. The current electrophysiological evidence concerning lead exposure and brain function in children, however, is too fragmentary to draw any firm conclusions. The use of evoked potential measures in prospective pediatric lead studies would provide a very useful adjunct to other neurobehavioral tests and would help to resolve current uncertainties regarding the neurobehavioral threshold of lead toxicity.

The adverse effects of lead on peripheral nerve function in children remain to be considered. Lead-induced peripheral neuropathies, although often seen in adults after prolonged exposures, are rare in children. Several articles (Anku and Harris, 1974; Erenberg et al., 1974; Seto and Freeman, 1964), however, describe case histories of children with lead-induced peripheral neuropathies, as indexed by electromyography, assessment of nerve conduction velocity, and observation of other overt neurological signs, such as tremor and wrist or foot drop. Frank neuropathic effects have been observed at blood lead levels of 60-80 µg/dl (Erenberg et al., 1974). In one case study (Seto and Freeman, 1964), signs indicative of peripheral neuropathy were reported to be associated with blood lead values of 30 µg/dl; however, lead lines in long bones suggested probable past exposures leading to peak blood lead levels at least as high as 40-60 µg/dl and probably in excess of 60 µg/dl (based on the data of Betts et al., 1973). In all of these case studies, some, if not complete, recovery of affected motor functions was reported after treatment for lead poisoning. A tentative association has also been hypothesized between sickle cell disease and increased risk of peripheral neuropathy as a consequence of childhood lead exposure. Half of the cases reported (10 out of 20) involved inner-city Black children, several with sickle cell anemia (Anku and Harris, 1974; Lampert and Schochet, 1968; Seto and Freeman, 1964; Imbus et al., 1978). In summary, evidence exists for frank peripheral neuropathy in children, and such neuropathy can be associated with blood lead levels at least as low as 60 µg/dl and, possibly, as low as 40-60 µg/dl.

Further evidence for lead-induced peripheral nerve dysfunction in children is provided by two studies by Feldman et al. (1973a,b, 1977) of inner city children and from a study by Landrigan et al. (1976) of children living in close proximity to a smelter in Idaho. The nerve conduction velocity (NCV) results from the latter study are presented in Figure 12-4 in the form of a scatter diagram relating peroneal nerve conduction velocities to blood lead levels. No clearly abnormal conduction velocities were observed, although a statistically significant negative correlation was found between peroneal NCV and blood lead levels ($r = -0.38$, $p < 0.02$ by one-tailed t-test). These results, therefore, provide evidence for significant slowing of nerve conduction velocity (and, presumably, for advancing peripheral neuropathy as a function of increased blood lead levels), but do not allow clear statements regarding a threshold for pathologic slowing of NCV.

In a recent study mentioned earlier, Winneke et al. (1984) evaluated neurophysiological functions as well as neuropsychologic performance in children from Nordenham, FRG. Results from a standard neurological examination and sensory nerve conduction velocities of the radial and median nerves were analyzed in relation to concurrent blood lead values and umbilical cord blood lead levels sampled approximately six years earlier. Contrary to expectations, increasing conduction velocities for radial and median nerves were found to be significantly

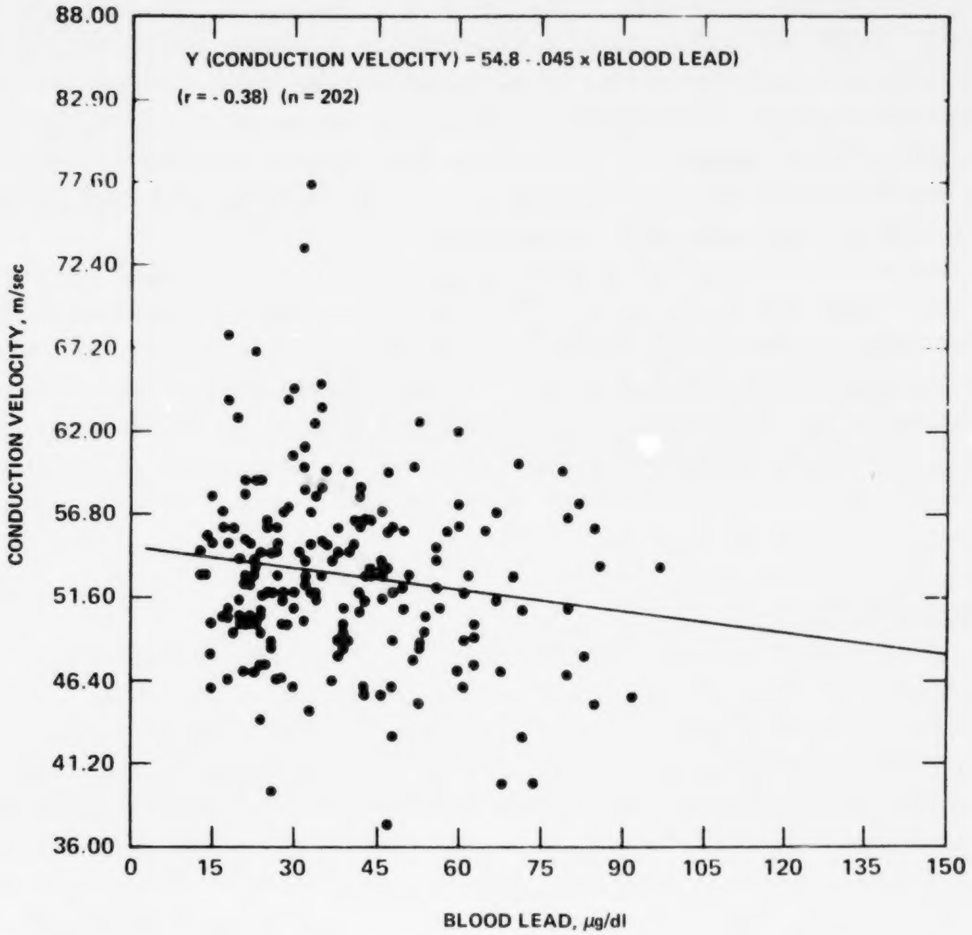


Figure 12-4. Peroneal nerve conduction velocity versus blood lead level, Idaho, 1974.

Source: Landrigan et al. (1976).

associated with current blood lead levels (at p <0.01 and <0.10, respectively). As noted above, visual evoked potentials showed a significantly decreased latency in one component, which suggested more rapid conduction in the visual pathway, consistent with the peripheral nerve conduction findings. Somatosensory evoked potentials showed no significant effect; nor were associations found between any of the electrophysiological measures and cord blood lead levels or any of a number of socio-hereditary background variables (the latter of which were strongly related to neuropsychologic outcome results).

The lead-associated increases in nerve conduction observed by Winneke et al. (1984) for children with blood lead levels below 25-30 μg/dl differ from previously noted findings of slowed NCVs being associated with increasing blood lead values above 30 μg/dl. However, the apparently paradoxical findings were noted by the investigators as being consistent with those of Englert (1978), who similarly found an increase in the motor NCV of the median nerve among lead-exposed children in Nordenham. Winneke et al. (1984) nevertheless cautioned that these findings still require experimental confirmation before a bi-phasic effect of lead on peripheral nervous functions can be assumed.

12.4.3 Animal Studies

The following sections focus on recent experimental studies of lead effects on behavioral, morphological, physiological, and biochemical parameters of nervous system development and function in laboratory animals. Several basic areas or issues are addressed: (1) behaviorial toxicity, including the question of critical exposure periods for concurrent induction or later expression of behavioral dysfunction in motor development, learning performance, and social interactions; (2) alterations in morphology, including synaptogenesis, dendritic development, myelination, and fiber tract formation; (3) perturbations in various electrophysiological parameters, e.g., ionic mechanisms of neurotransmission or nerve conduction velocities in various tracts; (4) disruptions of biochemical processes such as energy metabolism and chemical neurotransmission; (5) the persistence or reversibility of the above types of effects beyond the cessation of external lead exposure; and (6) the issue of "threshold" for neurotoxic effects of lead.

Since the initial description of lead encephalopathy in the developing rat (Pentschew and Garro, 1966), considerable effort has been made to define more closely the extent of nervous system involvement at subencephalopathic levels of lead exposure. This experimental effort has focused primarily on exposure of the developing organism. The interpretation of a large number of studies dealing with early *in vivo* exposure to lead has, however, been made difficult by variations in certain important experimental design factors across available studies.

One of the more notable of the experimental shortcomings of some studies has been the occurrence of undernutrition in experimental animals (U.S. Environmental Protection Agency, 1977). Conversely, certain other studies of lead neurotoxicity in experimental animals have been confounded by the use of nutritionally fortified diets, i.e., most commercial rodent feeds (Michaelson, 1980). In general, deficiencies of certain minerals result in increased absorption of lead, whereas excesses of these minerals result in decreased uptake (see Chapter 10). Dietary mineral and vitamin components are known to alter certain neurotoxic effects of lead (Woolley and Woolley-Efigenio, 1983). Commercial feeds may also be contaminated by variable amounts of heavy metals, including as much as 1.7 ppm of lead (Michaelson, 1980). Questions have also been raised about possible nutritional confounding due to the acetate radical in the lead acetate solutions often used as the source of lead exposure in experimental animal studies (Barrett and Livesey, 1982).

Another important factor that varies among many studies is the route of exposure to lead. Exposure of the suckling animal via the dam would appear to be the most "natural" method, yet may be confounded by lead-induced chemical changes in milk composition. On the other hand, intragastric gavage allows one to determine precisely the dose and chemical form of administered lead, but the procedure is quite stressful to the animal and does not necessarily reflect the actual amount of lead absorbed by the gut. Injections of lead salts (usually performed intraperitoneally) do not mimic natural exposure routes and can be complicated by local tissue calcinosis at the site of repeated injections.

Another variable in experimental animal studies that merits attention concerns species and strains of experimental subjects used. Reports by Mykkänen et al. (1980) and Overmann et al. (1981) have suggested that hooded rats and albino rats may differ in their sensitivity to the toxic effects of lead, possibly because of differences in their rates of maturation and/or rates of lead absorption. Such differences may account for variability of lead's effects and differences in exposure-response relationships between different species as well.

Each of the above factors may lead to widely variable internal lead burdens in the same or different species exposed to roughly comparable amounts of lead, making comparison and interpretation of results across studies difficult (Shellenberger, 1984). The force of this discussion, then, is to emphasize the importance of measurements of blood and tissue concentrations of lead in experimental studies. Without such measures, attempts to formulate dose-response relationships are futile. This problem is particularly evident in later sections dealing with the morphological, biochemical, and electrophysiological aspects of lead neurotoxicity. *In vitro* studies reviewed in those sections, in contrast to *in vivo* studies, are of limited relevance in dose-response terms. The *in vitro* studies, however, provide valuable information on basic mechanisms underlying the neurotoxic effects of lead.

The following sections discuss and evaluate the most recent studies of nervous system involvement at subencephalopathic exposures to lead. Most of the older studies are reviewed in the previous Air Quality Criteria Document for Lead (U.S. Environmental Protection Agency, 1977).

12.4.3.1 The Behavioral Toxicity of Lead: Critical Periods for Induction and Expression of Effects. The perinatal period of ontogeny has been generally recognized as a particularly critical time for the initiation of neurobehavioral pertubations by exposure to lead (U.S. Environmental Protection Agency, 1977; Reiter, 1982; Kimmel, 1984). This view is based in part on the metabolic characteristics of young organisms, which show comparatively greater absorption and retention of lead (see Chapter 10). In addition, a number of behavioral studies have compared the effects of lead exposure at different times during ontogeny and have often found effects associated only with perinatal exposure (e.g., Brown, 1975; Brown et al., 1971; Padich and Zenick, 1977; Shigeta et al., 1977; Snowdon, 1973).

On the other hand, several studies have demonstrated that alterations in behavior can result from exposure after weaning or maturation in rats (Angell and Weiss, 1982; Bushnell and Levin, 1983; Cory-Slechta and Thompson, 1979; Geist and Mattes, 1979; Geist et al., 1985; Kowalski et al., 1982; Lanthorn and Isaacson, 1978; McLean et al., 1982; Nation et al., 1982; Ogilvie and Martin, 1982; Shapiro et al., 1973). Similar findings have been noted in adult subjects of other species, including pigeons (Barthalmus et al., 1977; Dietz et al., 1979) and fish (Weir and Hine, 1970).

The fact that late developmental exposure to lead can induce behavioral effects in animals does not mean, of course, that early exposure is less effective or important. As the following sections will show, the toxic effects of lead may be induced at various stages of life, with the expression of these effects following closely or, in some cases, after considerable delay.

12.4.3.1.1 Development of motor function and reflexes. A variety of methods have been used to assess the effect of lead on the ability of experimental animals to respond appropriately, either by well-defined motor responses or gross movements, to a defined stimulus. Such responses have been variously described as reflexes, kineses, taxes, and "species-specific" behavior patterns. The air righting reflex, which refers to the ability to orient properly with respect to gravity while falling through the air and to land on one's feet, is only one of several commonly used developmental markers of neurobehavioral function (Tilson and Harry, 1982). Overmann et al. (1979) found that development of this particular reflex was slowed in rat pups exposed to lead via their dams (0.02 or 0.2 percent lead acetate* in the dams' drinking water). However, neither the auditory startle reflex nor the ability to hang suspended by the front paws was affected.

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*Concentrations are presented here as originally reported by authors. Note that a 0.2 percent solution of lead acetate contains 0.1 percent lead. Also, for comparative purposes, a concentration of 0.1 percent equals 1000 ppm.

Grant et al. (1980) exposed rats indirectly to lead in utero and during lactation through the mothers' milk and, after weaning, directly through drinking water containing the same lead concentrations their respective dams had been given. In addition to morphological and physical effects [see Sections 12.5, 12.6, and 12.8 for discussions of this work as reported by Fowler et al. (1980), Kimmel et al. (1980), Faith et al. (1979), and Luster et al. (1978)], there were delays in the development of surface righting and air righting reflexes in subjects exposed under the 50- and 250-ppm lead conditions; other reflexive patterns showed no effect. Locomotor development generally showed no significant alteration due to lead exposure, but body weight was significantly depressed for the most part in the 50- and 250-ppm pups.

Rabe et al. (1985) used a similar experimental paradigm to evaluate developmental landmarks in rat pups exposed via their dams to a 0.5 percent lead acetate solution. Although this concentration of lead was much higher than the drinking water solutions used by Grant et al. (1980), Rabe et al. (1985) found no apparent delays in the development of surface righting and negative geotaxis reflexes, nor in the age at which the pups' eyes opened. Body weight of the pups was reduced slightly at birth, but by postnatal day (PND) 30 the lead-exposed pups had attained normal average weight. In comparing these two studies, it should also be noted that the mean blood lead level at PND 16 was only 20 µg/dl for pups exposed indirectly to 2500 ppm lead by Rabe et al. (1985), as opposed to a median of 35 µg/dl at PND 11 for pups exposed indirectly to 50 ppm lead by Grant et al. (1980). These differences are probably attributable to the different diets used in the studies (see Mylroie et al., 1978).

The ontogeny of motor function in lead-exposed rat pups was also investigated by Overmann et al. (1981). Exposure was limited to the period from parturition to weaning and occurred through adulteration of the dams' drinking water with lead (0.02 or 0.2 percent lead acetate). The development of swimming performance was assessed on alternate days from PND 6 to 24. No alterations in swimming ability were found. Rotorod performance was also tested at PND 21, 30, 60, 90, 150, and 440. Overall, the ability to remain on a rotating rod was significantly impaired ($p < 0.01$) at 0.2 percent and tended to be impaired ($0.10 > p > 0.05$) at 0.02 percent (blood lead values were not reported). However, data for individual days were statistically significant only on PND-60 and 150. An adverse effect of lead exposure on rotorod performance at PND 30-70 was also found in an earlier study by Overmann (1977) at a higher exposure level of 30 mg/kg lead acetate by intubation (average blood lead value at PND 21: 173.5 ± 32.0 µg/dl). At blood lead concentrations averaging 33.2 ± 1.4 µg/dl, however, performance was not impaired. Moreover, other studies with average blood lead concentrations of approximately 61 µg/dl (Zenick et al., 1979) and 30-48 µg/dl (Grant et al., 1980) have not found significant effects of lead on rotorod performance when tested at PND 21 and 45, respectively. Comparisons between studies are confounded by differences in body weight and age at time of testing and by differences in speed and size of the rotorod apparatus (Zenick et al., 1979).

Kishi et al. (1983) evaluated reflex development and motor coordination in male rats exposed to lead acetate by gavage on PND 3-21. The air righting reflex was significantly delayed in all three lead exposure groups (the lowest level producing an average blood lead level of 59 µg/dl at PND 22). The startle reflex showed no effect, and eye opening was accelerated in the lowest exposure group. Rotorod performance at PND 53-58 was significantly impaired in the highest exposure group (average blood lead level: 186 µg/dl at PND 22). Ambulation was assessed at PND 59-60 and showed a high degree of variability across the lead exposure groups (very low or very high levels or movement); other measures of activity showed no differences. The effects on ambulation were not evident at PND 288-289.

Delays in the development of gross activity in rat pups have been reported by Crofton et al. (1980) and by Jason and Kellogg (1981). It should be noted that very few studies have been designed to measure the rate of development of activity. Ideally, subjects should be assessed daily over the entire period of development in order to detect any changes in the rate at which a behavior pattern occurs and matures. In the study by Crofton et al. (1980), photocell interruptions by pups as they moved through small passageways into an "exploratory cage" adjacent to the home cage were automatically counted on PND 5-21. Pups exposed in utero through the dams' drinking water (200 mg/l solution of lead chloride) lagged controls by approximately one day in regard to characteristic changes in daily activity count levels starting at PND 16. (Blood lead concentrations at PND 21 averaged 14.5 ± 6.8 µg/dl for representative pups exposed to lead in utero and 4.8 ± 1.5 µg/dl for controls.) Another form of developmental lag in gross activity around PND 15-18, as measured in an automated activity chamber, was reported by Jason and Kellogg (1981). Rats were intubated on PND 2-14 with lead at 25 mg/kg (blood lead: 50.07 ± 5.33 µg/dl) and 75 mg/kg (blood lead: 98.64 ± 9.89 µg/dl). In this case, the observed developmental lag was in the characteristic decrease in activity that normally occurs in pups at that age (Campbell et al., 1969; Melberg et al., 1976); thus, lead-exposed pups were significantly more active than control subjects at PND 18.

One question that arises when ontogenetic effects are discovered concerns the possible contribution of the lead-exposed dam to her offsprings' slowed development through, for example, reduced or impaired maternal caregiving behavior. A detailed assessment of various aspects of maternal behavior in chronically lead-exposed rat dams by Zenick et al. (1979), discussed more fully in Section 12.4.3.1.4, and other studies using cross-fostering techniques (Crofton et al., 1980; Mykkänen et al., 1980) suggest that the deleterious effects observed in young rats exposed to lead via their mothers' milk are not ascribable to alterations in the dams' behavior toward their offspring. Chronically lead-exposed dams may, if anything, tend to respond adaptively to their developmentally retarded pups by, for example, more quickly retrieving them to the nest (Davis, 1982) or nursing them for longer periods (Barrett and Livesey, 1983).

12.4.3.1.2 Locomotor activity. The spontaneous activity of laboratory animals has been measured frequently and in various ways as a behavioral assay in pharmacology and toxicology (Reiter and MacPhail, 1982). Such activity is sometimes described as gross motor activity or exploratory behavior, and is distinguished from the motor function tests noted in the previous section by the lack of a defined eliciting stimulus for the activity. With reports of hyperactivity in lead-exposed children (see Section 12.4.2), there has naturally been considerable interest in the spontaneous activity of laboratory animals as a model for human neurotoxic effects of lead (see Table 12-3). As a previous review (U.S. Environmental Protection Agency, 1977) of this material demonstrated, however, and as other reviews (e.g., Jason and Kellogg, 1980; Michaelson, 1980; Mullenix, 1980) have since confirmed, the use of activity measures as an index of the neurotoxic effects of lead has been fraught with difficulties.

First, there is no unitary behavioral endpoint that can be labeled "activity." Activity is, quite obviously, a composite of many different motor actions and can comprise diverse behavior patterns including (in rodents) ambulation, rearing, sniffing, grooming, and, depending on one's operational definition, almost anything an animal does. These various behavior patterns may vary independently, so that any gross measure of activity which fails to differentiate these components will be susceptible to confounding. Thus, different investigators' definitions of activity are critical to interpreting and comparing these findings. When these definitions are sufficiently explicit operationally (e.g., activity as measured by rotations of an "activity wheel"), they are frequently not comparable with other operational definitions of activity (e.g., movement in an open field as detected by photocell interruptions). Moreover, empirical comparisons (e.g., Capobianco and Hamilton, 1976; Tapp, 1969) show that different measures of activity do not necessarily correlate with one another quantitatively.

In addition to these rather basic difficulties, activity levels are influenced greatly by numerous variables such as age, sex, estrous cycle, time of day, novelty of environment, and food deprivation. If not controlled properly, any of these variables can confound measurements of activity levels. Also, nutritional status has been a frequent confounding variable in experiments examining the neurotoxic effects of lead on activity (see reviews by U.S. Environmental Protection Agency, 1977; Jason and Kellogg, 1980; Michaelson, 1980). In general, it appears that rodents exposed neonatally to sufficient concentrations of lead experience undernutrition and subsequent retardation in growth; as Loch et al. (1978) have shown, retarded growth *per se* can induce increased activity of the same type that has been attributed to lead alone in some earlier studies.

In view of the various problems associated with the use of activity measures as a behavioral assay of the neurotoxic effects of lead, the discrepant findings summarized in Table

TABLE 12-3. EFFECTS OF LEAD ON ACTIVITY IN RATS AND MICE

Increased	Decreased	Age-dependent, qualitative, mixed or no change
Baraldi et al. (1985)	Booze et al. (1983)	Alfano and Petit (1985)
Czech and Hoium (1984)	Driscoll and Stegner (1976)	Barrett and Livesey (1982, 1985)
Driscoll and Stegner (1978)	Flynn et al. (1979)	Brown (1975)
Golter and Michaelson (1975)	Gray and Reiter (1977)	Collins et al. (1984)
Kostas et al. (1976)	Reiter et al. (1975)	Crofton et al. (1980)
Overmann (1977)	Verlangieri (1979)	Dolinsky et al. (1981)
Petit and Alfano (1979)		Dubas and Hrdina (1978)
Sauerhoff and Michaelson (1973)		Geist and Balko (1980)
Silbergeld and Goldberg (1973, 1974a,b)		Geist and Praed (1982)
Weinreich et al. (1977)		Grant et al. (1980)
Winneke et al. (1977)		Gross-Selbeck and Gross-Selbeck (1981)
		Hastings et al. (1977)
		Jason and Kellogg (1981)
		Kishi et al. (1983)
		Kostas et al. (1978)
		Krehbiel et al. (1976)
		Loch et al. (1978)
		McCarren and Eccles (1983)
		Minsker et al. (1982)
		Mullenix (1980)
		Ogilvie and Martin (1982)
		Rabe et al. (1985)
		Rafales et al. (1979)
		Schlipköter and Winneke (1980)
		Shimojo et al. (1983)
		Sobotka and Cook (1974)
		Sobotka et al. (1975)
		Zimering et al. (1982)

12-3 should come as no surprise. Until the measurement of "activity" can be better standardized, there appears to be little basis for comparing, or utility in further discussing, the results of studies listed in Table 12-3.

12.4.3.1.3 Learning ability. When animal learning studies related to the neurotoxic effects of lead were reviewed in 1977 (U.S. Environmental Protection Agency, 1977), a number of criticisms of existing studies were noted. A major limitation of early work in this field was the lack of adequate information on the exposure regimen (dosage of lead, how precisely administered, timing of exposure) and the resulting body burdens of lead in experimental subjects (concentrations of lead in blood, brain, or other tissue; time course of blood or tissue lead levels; etc.). A review of studies appearing since 1977 reveals a notable improvement in this regard. A number of more recent studies have also attempted to control for the confounding factors of litter effects and undernutrition--variables that were generally not controlled in earlier studies.

Unfortunately, other criticisms are still valid today. The reliability and validity of behavioral assays remain to be established adequately, although progress is being made. The reliability of a number of common behavioral assays for neurotoxicity is currently being determined by several independent U.S. laboratories (Buelke-Sam et al., 1985). The results of this program should help standardize some behavioral testing procedures and perhaps create some reference methods in behavioral toxicology. Also, as well-described studies are replicated within and between laboratories, the reliability of certain experimental paradigms for demonstrating neurotoxic effects is effectively established.

Some progress is also being made in dealing with the issue of the validity of animal behavioral assays. As the neurological and biochemical mechanisms underlying reliable behavioral effects become better understood, the basis for extrapolating from one species to another becomes stronger and more meaningful. An awareness of different species' phylogenetic, evolutionary, and ecological relationships can also help elucidate the basis for comparing behavioral effects in one species with those observed in another (Davis, 1982).

Tables 12-4 and 12-5 summarize exposure conditions, testing conditions, and results of a number of recent studies of animal learning (see U.S. Environmental Protection Agency, 1977, for a summary of earlier studies). The variety of exposure measures and testing paradigms makes it impossible to organize these studies in a coherent dose-response fashion. Consequently, the tables present, respectively, rodent and primate studies arranged alphabetically by author and/or chronologically where appropriate. One point of obvious interest is the lowest level of exposure at which behavioral effects are clearly evident. Such a determination is best done on a species-by-species basis. Rats seem to be the experimental animal

TABLE 12-4. RECENT ANIMAL TOXICOLOGY STUDIES OF LEAD'S EFFECTS ON LEARNING IN RODENTS[a]

Reference	Experimental animal (species or strain)	Lead exposure conc. (medium)	Lead exposure period (route)	Treatment groups (n)	Litters per group	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
Alfano and Petit (1985)	Rat (L-E)	0.4 or 4% $PbCO_3$ (food)	PND 1-25 (via dam and direct)	C_5[b] (40) C_{10} (50) Pb_1 (50) Pb_2 (50)	8 5 5 10	See Petit & Alfano (1979) for representa- PbB levels	66-100 d 101-123 d	Passive avoidance (remain in 1 of 2 compartments to avoid shock); T-maze (spontaneous alternation)	B.w. of C_5-Ss > C_0- and Pb_2-Ss	Latencies of Pb-Ss sig. shorter than C-Ss'; Pb_2 latencies sig. shorter than C_{10}'s. Both Pb groups performed sig. less spontaneous alternation than C-Ss.
Angell & Weiss (1982)	Rat (L-E)	0.2% $Pb(Ac)_2$ (water)	PND 3-21 (dam's milk) and/or 21-130 (direct)	0-0 (20) 0-Pb (20) Pb-0 (24) Pb-Pb (24)	} 5, split } 6, split	PbB (130d): 0-0: 2 µg/dl 0-Pb: 66 Pb-0: 9 Pb-Pb: 64	58-130 d	Operant (multiple FI-TO-FR-TO)	Pb-Pb Ss sig. lower b.w. post-weaning	Groups exposed post-weaning (0-Pb, Pb-Pb) had longer Inter-Response Times; group exposed preweaning (Pb-0) had shorter IRTs.
Booze et al. (1983)	Rat (F-344)	3 or 6 mg/kg TEL (15% ethanol)	PND 5, once (s.c.)	C_0[c] (24) C_{15} (23) Pb_1 (24) Pb_2 (23)	random selection from 12 litters	?	18 d	Passive avoidance (remain in 1 of 2 compartments to avoid shock)	B.w. of Pb-Ss < C-Ss	Pb_1-females showed sig. poorer retention of avoidance than ethanol controls.
Bushnell and Levin (1983)	Rat (S-D)	10 or 100 ppm Pb (water)	PND 21-56 (direct)	C (6) Pb_1 (6) Pb_2 (6)	?	Brain-Pb[d] (57 d): C: 0 µg/g Pb_1: 0.05 Pb_2: 0.70	4, 5, 6, and 7 wk	Radial arm maze (spontaneous alternation)	B.w. of Pb_2-Ss < C-Ss	Both Pb_1- and Pb_2-Ss chose unexplored arms sig. less often than C-Ss.

TABLE 12-4. (continued)

Reference	Experimental animal (species or strain)	Lead exposure conc. (medium)	Lead exposure period (route)	Treatment groups (n)	Litters per group	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
Cory-Slechta & Thompson (1979)	Rat (S-D)	1) 50, 2) 300, or 3) 1000 ppm $Pb(Ac)_2$ (water)	PND 20- a) 70 or b) 150 (direct)	1a: C $(4)^e$ Pb (5) 1b: C $(4)^e$ Pb (6) 2b: C $(3)^e$ Pb (4) 3b: C $(4)^e$ Pb (5)	random assign-ment	PbB (150 d): C: ~6 μg/dl 1a: ~3 1b: ~7 2b: ~27 3b: ~42	55-140 d	Operant (FI-30 sec)	None	Increased response rate and inter-S variability in both $Pb_{1a,b}$ and Pb_2 groups; decreased response rate in Pb_3 group; effects in Pb_{1a} reversed after exposure terminated.
Cory-Slechta et al. (1981)	Rat (S-D)	100 or 300 ppm $Pb(Ac)_2$ (water)	PND 21-?	C (4) Pb_1 (5) Pb_2 (5)	random assign-ment	Brain-Pb (post-test): C: 14-26 ng/g Pb_1: 40-142 Pb_2: 320-1080	55-? d	Operant (minimum duration bar-press)	None	Pb groups impaired: decreased response durations; increased response latencies; failure to improve performance by external stimulus control.
Cory-Slechta et al. (1983)	Rat (L-E)	50, 100 or 500 $Pb(Ac)_2$ (water)	PND 21- a) 158 or b) 315 (direct)	C (6) Pb_1 (6) Pb_2 (6) Pb_3 (6)	random assign-ment, balanced for b.w.	PbB (max): C: <2 μg/dl Pb_1: ~40 Pb_2: ~50 Pb_3: ~90 Brain-Pb (336 d): C: 0.01 μg/g Pb_1: 0.31 Pb_2: 0.57 Pb_3: 1.4	55 d	Operant (FI-1 min)	None	Higher response rates in Pb-Ss; number of sessions to reach max. rate a direct function of Pb expos. Early vs. late termination of exposure period produced no difference in response rates. sponse rates.

TABLE 12-4. (continued)

Reference	Experimental animal (species or strain)	Lead exposure		Treatment groups (n)	Litters per group	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
		conc. (medium)	period (route)							
Cory-Slechta et al. (1985)	Rat (L-E)	25 ppm Pb $(Ac)_2$ (water)	PND 21-termination (direct)	C (12) Pb (12)	random assignment, balanced for weight	PbB (99, 143, 186 d): C: <1 µg/dl Pb: 15-20	50 d	Operant (FI-1 min)	None	Sig. higher response rate and shorter IRTs by Pb-Ss during first 40 sessions.
Dietz et al. (1978)	Rat Expt. 1(L-E)	200 mg/kg $Pb(Ac)_2$ (gavage)	PND 3-30 (direct)	C (6) Pb (7)}	2, split	?	3 mo or 21 mo	Operant (minimum 20-sec between bar-presses)	None	Short IRTs (≤4 sec) more prevalent in Pb-Ss than in C-Ss, but did not result in different reward rates; Pb-Ss showed higher variability in response rate under d-amphetamine treatment.
	Expt. 2(CD)	250 ppm Pb (water)	Preconception to termination (via dam until weaning, then direct)	C $(4)^e$ Pb (4)	?	?	8 mo		Pb-Ss b.w. lower 1 wk. prior to test; C-Ss reduced to same wt. at test.	
Flynn et al. (1979)	Rat (L-E) Expt. 1	0.5% $Pb(Ac)_2$ (water)	Preconception - PND 22 (via dam)	C (8) Pb (10)	8 10	Brain-Pb (3 d): C: ~0 Pb: 0.174 µg/g (30-34 d): no sig. diffs.	?	Radial arm maze (spontaneous alternation)	Brain wts. of Pb-Ss < C-Ss; no other differences.	No sig. difference between Pb-Ss and C-Ss.
	Expt. 2	0.2% $Pb(Ac)_2$ (water), 225 mg/kg Pb (gavage), 0.25% $Pb(Ac)_2$ (water)	Preconception - birth (via dam), birth - weaning (direct), weaning - termination (direct)	C (12) Pb (12)	6 6	(75-76 d): C: 0.13 µg/g Pb: 1.85	49-58 d	Passive avoidance (remain in 1 of 2 compartments to avoid shock)	None	No sig. difference in trials to criterion, but Pb-Ss made sig. fewer partial excursions from "safe" compartment.
	Expt. 3	same as above except 90 mg/kg Pb (gavage)	same as above except stopped at PND 33	C (10) Pb (10)}	4	see above	58-60 d	Shuttle-box signalled avoidance (move from one compartment to other to avoid elect. shock)	None	No sig. difference between Pb-Ss and C-Ss.

TABLE 12-4. (continued)

Reference	Experimental animal (species or strain)	Lead exposure		Treatment groups (n)	Litters per group	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
		conc. (medium)	period (route)							
Geist & Mattes (1979)	Rat (S-D)	25 or 50 ppm Pb(Ac)$_2$ (water)	PND 23-termination (direct)	C (7) Pb$_1$ (7) Pb$_2$ (7)	?	?	58-? d	Hebb-Williams maze (find way to goal box)	None	Pb$_1$- and Pb$_2$-Ss made sig. more errors than C-Ss; Pb$_2$-Ss slower than C-Ss to traverse maze.
Geist et al. (1985)	Rat (S-D)	25 or 50 ppm Pb(Ac)$_2$ (water)	PND 21-65 (direct)	C (6) Pb$_1$ (6) Pb$_2$ (6)	?	?	61 d 143 d	T-maze (spontaneous alternation); Hebb-Williams maze (find way to goal box)	None	Rate of spontaneous alternation sig. reduced in Pb-Ss. No sig. differences in H-W maze except except for shorter latency of Pb-Ss to leave start box.
Gross-Selbeck & Gross-Selbeck (1981)	Rat (W) F$_1$	1 g/kg Pb(Ac)$_2$ (food)	Postweaning - termination (direct)	C (6) Pb (6)	?	PbB (~180 d): C: 6.2 μg/dl Pb: 22.7	Adult	Operant (DRH)	None	Both F$_1$ and F$_2$ (especially F$_2$) Pb-Ss had greater % rewarded responses than C-Ss, i.e., Pb-Ss bar-pressed at higher rate than C-Ss.
	F$_2$	"	Preconception - weaning (via dam)	C (6) Pb (6)	?	(~110 d): C: 3.7 Pb: 4.6	3-4 mo	"	"	
Hastings et al. (1977)	Rat (L-E)	109 or 545 ppm Pb(Ac)$_2$ (water)	PND 0-21 (dam's milk)	C (12) Pb$_1$ (12) Pb$_2$ (12)	random selection from 9 litters	PbB (20 d): C: 11 μg/dl Pb$_1$: 29 Pb$_2$: 42 (60 d): C: 4 Pb$_1$: 5 Pb$_2$: 9	~90-186 d	Operant (successive brightness discrim.)	None	No sig. differences between Pb-Ss and C-Ss in learning original or reversed discrim. task.

TABLE 12-4. (continued)

Reference	Experimental animal (species or strain)	Lead exposure conc. (medium)	Lead exposure period (route)	Treatment groups (n)	Litters per group	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
Hastings et al. (1979)	Rat (L-E)	0.02 or 0.2% $Pb(Ac)_2$ (water)	PND 0-21 (dam's milk)	C (23) Pb_1 (11) Pb_2 (13)	random selection from 15 litters	PbB (20 d): C: 11 µg/dl Pb_1: 29 Pb_2: 65 Brain-Pb (20 d): C: 12.5 µg% Pb_1: 29 Pb_2: 65	120 d 270 d 330 d	1) Operant (simult. vis. discrim.) 2) T-maze (success. vis. discrim.) 3) Operant (go/no-go task)	None	Pb_2-Ss sig. slower to reach criterion than C-Ss on simultaneous visual discrimination task; no sig. differences on successive and go/no-go discrim. tasks.
Hastings et al. (1984)	Rat (L-E)	0.10% or 0.20% $Pb(Ac)_2$ (water)	PND 0 to a) 30 (dam's milk) or b) termination (direct)	C (22) Pb_1 (25) Pb_{2a} (22) Pb_{2b} (23)	random selection from 49 litters	PbB (20 d): C: 3 µg/dl Pb_1: 30 Pb_{2a}: 57 Pb_{2b}: 40 (90 d): C: 5 Pb_1: 31 Pb_{2a}: 9 Pb_{2b}: 42	~90 d	Operant (1) spatial discr. with successive reversals; 2) simult. visual discr.)	None	No sig. differences in performance except for sig. pos. correlation between day-20 PbB levels and number of non-rewarded responses between trials.
Kishi et al. (1983)	Rat (W)	45, 90, or 180 µg/g b.w. (water)	PND 3-21 (gavage)	C (10) Pb_1 (10) Pb_2 (10) Pb_3 (9)	random selection	PbB (22d): C: 10 µg/dl Pb_1: 59 Pb_2: 152 Pb_3: 186	75-270 d	Operant (1) CRF 2) FR 20 3) EXT 4) DRL 20-sec 5) EXT)	B.w. of Pb_3-Ss < C-Ss	Sig. greater variability in CRF responding by Pb_1 and Pb_3-Ss. No sig. differences in mean response rates except Pb_1-Ss better than C-Ss at end of DRL training.
Kowalski et al. (1982)	Mouse (W)	2 ppm Pb (water)	Adult (direct)	C (16) Pb (16)	?	?	Adult (13 d after start of exposure)	Water T-maze (spatial discrim.)	None	Pb-Ss made more errors than C-Ss; food deprivation exacerbated effect.

TABLE 12-4. (continued)

Reference	Experimental animal (species or strain)	Lead exposure		Treatment groups (n)	Litters per group	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
		conc. (medium)	period (route)							
Lanthorn & Isaacson (1978)	Rat (L-E)	0.27% Pb (water)	Adult (direct)	C (4) Pb (6)	?	?	Adult	T-maze (1) spontaneous alternation 2) light discrim. 3) spatial discrim.)	C-Ss pair-fed to control for loss of b.w.	Pb-Ss had sig. lower rate of spontaneous alternation; Pb-Ss sig. slower than C-Ss only on 1st spatial discrim. task.
McLean et al. (1982)	Mouse (Swiss)	20 or 2000 ppm Pb (water)	Adult (direct)	C (16) Pb_1 (16) Pb_2 (16)	?	?	Adult (10 d after start of exposure)	Water T-maze (spatial discrim.)	None	Pb-Ss showed no improvement in performance compared to C-Ss.
Milar et al. (1981b)	Rat (L-E)	25, 100, or 200 mg/kg b.w. Pb (gavage)	PND 4-31 (direct)	C (10) Pb_1 (5) Pb_2 (4) Pb_3 (6)	3 4 4 4	PbB (32 d): C: 5 µg/dl Pb_1: 26 Pb_2: 63 Pb_3: 123	50 d	Operant (spatial alternation levers)	Sig. slower growth rate in Pb_3-Ss	No sig. differences between C-Ss and Pb-Ss.
Nation et al. (1982)	Rat (S-D)	10 mg/kg b.w. Pb (food)	PND 100-termination (direct)	C (8) Pb (8)	?	?	156 d	Operant (conditioned suppression of responding on multiple VI schedule)	None	Presentation of tone associated with electrical shock disrupted steady-state responding more in PB-Ss than in C-Ss.
Overmann (1977)	Rat (L-E)	10, 30, or 90 $Pb(Ac)_2$ (gavage)	PND 3-21 (direct)	C Pb_1 12- Pb_2 25 Pb_3 ea.	?	PbB (21 d): C: 15 µg/dl Pb_1: 33.2 Pb_2: 173.5 Pb_3: 226.1	26-29 d 67-89 d 79-101 d 83-105 d 95-117 d	Aversive conditioning (1) active 2) passive) Operant (inhibit response) E-maze discrim.: (1) spatial 2) tactile 3) visual)	None	Pb_3-Ss sig. slower in acquisition and extinction of active avoidance response; no sig. diffs. for passive avoidance. All Pb groups failed to inhibit responses $Pb_{2,3}$-Ss sig. worse than C-Ss only on tactile discrim.

TABLE 12-4. (continued)

Reference	Experimental animal (species or strain)	Lead exposure		Treatment groups (n)	Litters per group	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
		conc. (medium)	period (route)							
Padich and Zenick (1977)	Rat (CD)	750 mg/kg $Pb(Ac)_2$ (water)	Preconception to a) weaning (via dam) or b) termination (via dam and direct); or Weaning to termination (direct only)	0-0 (10) 0-Pb (10) Pb-0 (10) Pb-Pb (10)	5 5 5 5	?	42-? d	Operant (FR 20)	B.w. of Pb-Ss < 0-Ss from birth to weaning.	Pb-Pb group had sig. fewer rewarded responses and took sig. longer to complete FR 20 requirement.
Penzien et al. (1982)	Rat (albino)	50 mg/kg b.w. $Pb(Ac)_2$ (?)	3 times over 5 days (i.p.)	C (4) Pb (7)	?	?	?	Lashley III maze (find way to goal box)	B.w. loss, impaired gait, slowed movement, some ataxia of hind limbs	Pb-Ss showed slower acquisition (in terms of speed and rate, but not errors); by 3rd session, no sig. diffs. except that Pb-Ss made sig. fewer errors than C-Ss.
Petit & Alfano (1979)	Rat (L-E)	0.4 or 4% $PbCO_3$ (food)	PND 1-25 (via dam and direct)	C_i (22) C_e (22) Pb_{1i} (22) Pb_{1e} (22) Pb_{2i} (22) Pb_{2e} (22)	~7 each; split for "i" (isolation and "e" (enrichment) conditions	PbB (25 d): C: 2 μg/dl Pb_1: 331 Pb_2: 1297	66-115 d	Hebb-Williams maze (find way to goal box) Passive avoidance (remain in compartment to avoid shock)	B.w. of Pb_2-Ss < C-Ss, Pb_1-Ss > C-Ss; gross toxicity in Pb_2-Ss; lower brain wts. in Pb_1-Ss	No sig. diff. between Pb- and C-Ss in maze learning; isolation-reared Pb-Ss less successful than C_i-Ss on passive-avoidance task; enrichment-reared Pb_1-Ss = C_e-Ss but Pb_{2e}-Ss sig. worse on passive avoidance.
Rabe et al. (1985)	Rat (L-E)	0.5% $Pb(Ac)_2$ (water)	Preconception to birth (via dam)	C_{1f}(16) C_2 (12) Pb (14)	2 each, split	PbB (1 d): $C_{1,2}$: 10 μg/dl Pb: 98 (16 d): $C_{1,2}$: 9 Pb: 20	17 d	T-maze (spatial discr., reversal)	B.w. of Pb-Ss < C-Ss at birth (includes pair-fed C_2-Ss)	No sig. diffs. in acquisition or reversal errors.

TABLE 12-4. (continued)

Reference	Experimental animal (species or strain)	Lead exposure		Treatment groups (n)	Litters per group	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
		conc. (medium)	period (route)							
Rosen et al. (1985)	Rat (L-E)	10 mg/kg b.w. $Pb(Ac)_2$ (?)	PND 1-20, daily (i.p.)	C_1 (16) Pb_1 (15) C_2 (8) Pb_2 (8)	4, split 2, split	PbB (21 d): C_1: 3 µg/dl Pb_1: 158 (180 d): C_2: 5 Pb_2: 8	1) 30-50 d and/or 2) 150 d	Radial-arm maze (find food in each of 8 arms); passive avoidance (remain in 1 of 2 compartments to avoid shock)	B.w. of Pb-Ss < C-Ss at 25 d, but not sig. after 50 d	No sig. differences on radial arm maze for either Pb_1 (young) or Pb_2 (adult) Ss. Sig. longer latency on passive avoidance for Pb_1-Ss, but not when retested as adults. Pb_2-Ss tested first time at 150 d had sig. shorter latencies (i.e., performed worse than C-Ss).
Schlipköter & Winneke (1980)	Rat (?) Expt. 1	25 ppm Pb (food)	Preconception - PND 120 (via dam and direct)	C (10) Pb_1 (18)	?	PbB: all C: <5 µg/dl Pb_1 (21 d): 39.5 (4 mo): 12.0	7 mo	Lashley jumping stand (size discrim.)	?	Sig. increase in error repetition by Pb_1-Ss.
	Expt. 2	75 ppm Pb (food)	a) Prenatal-7 mo (via dam and direct b) Prenatal-weaning (via dam)	C (10) Pb_{2a} (10) Pb_{2b} (10)	?	Pb_{2a}: (21 d) 29.2 (7 mo) 27.0 Pb_{2b}: (21 d) 29.2 (7 mo) 5.2	"	"	?	Non-sig. ($p < 0.10$) increase in error repetition by Pb_2-Ss.
	Expt. 3	-----Same as Expt. 2-----		C (14) Pb_{3a} (14) Pb_{3b} (14)	?	Pb_{3a}: (21 d) 29.9 (7 mo) 30.8 Pb_{3b}: (21 d) 29.9 (7 mo) 1.8	"	"	?	No sig. differences between Pb_3-Ss and C-Ss.
	Expt. 4	25 or 75 ppm Pb (food)	Prenatal - 7 mo (via dam and direct)	C (10) Pb_{4a} (10) Pb_{4b} (10)	?	(120 d) Pb_{4a}: 17.8 Pb_{4b}: 28.6	"	Water maze (spatial discrim.)	?	35% of Pb_4-Ss failed to reach criterion (vs. 10% C-Ss); 35% also failed retest after 1 wk (vs. 0% C-Ss).

TABLE 12-4. (continued)

Reference	Experimental animal (species or strain)	Lead exposure conc. (medium)	Lead exposure period (route)	Treatment groups (n)	Litters per group	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
Taylor et al. (1982)	Rat (CD)	200 or 400 mg/l $Pb(Ac)_2$ (water)	Preconception - weaning (via dam)	C (12) Pb_1 (16) C_2 (4) Pb_2 (4)	6^g 8^g 2^g 2^g	PbB (21 d): C: 3.7 μg/dl Pb_1: 38.2 Pb_2: 49.9	11 d	Runway (traverse alley to reach dam and dry suckle)	None	No sig. differences in acquisition of response, but both Pb groups sig. slower to extinguish when response no longer rewarded.
Winneke et al. (1977)	Rat (W)	1.38 g $Pb(Ac)_2$ per kg diet (food)	Preconception - testing (via dam and direct)	C (20) Pb (20)	? (random selection from 110 male pups)	PbB (~16 d): C: 1.7 μg/dl Pb: 26.6 (~190 d) Pb: 28.5	100-200 d	Lashley jumping stand (visual discrim. of stimulus: 1)orientation 2)size)	B.w. of Pb-Ss > C-Ss; however, size of Pb-Ss litters < C-S litters.	Pb-Ss sig. slower to learn size discrimination; no difference between Pb and C groups on orientation discrim. (a relatively easy task).
Winneke et al. (1982b)	Rat (W) Expt. 1	80, 250 or 750 ppm Pb (food)	Preconception - testing (via dam and direct)	C (16) Pb_1 (16) Pb_2 (16) Pb_3 (16)	random selection from 5-6 litters per condition	?	70-100 d	Shuttle-box signalled avoidance (move from one compartment to avoid elect. shock)	ALA-D at 90 d: C: 7.05 U/l Pb_1: 4.26 Pb_2: 1.92 Pb_3: 1.18	Expt. 1 Pb-Ss sig. faster than C-Ss to learn avoidance response.
	Expt. 2	-Continuation of Expt. 1-		C (10) Pb_2 (10) Pb_3 (10)	(females dropped; no Pb_1 group for Expt. 2)	?	190-250 d	Lashley jumping stand (size discrim.)		Expt. 2 Pb-Ss sig. slower than C-Ss to learn size discrim.
Zenick et al. (1978)	Rat (CD)	1000 mg/kg $Pb(Ac)_2$ (water)	Preconception - weaning (via dam)	C (10) Pb (10)	5 5	?	30-40 d 55-63 d	Water T-maze (1) black-white discrim. 2) shape discrim.)	B.w. of Pb-Ss < C-Ss from birth to 50 d.	On both discrim. tasks, Pb-Ss made sig. more errors with sig. shorter response.

TABLE 12-4. (continued)

Reference	Experimental animal (species or strain)	Lead exposure conc. (medium)	Lead exposure period (route)	Treatment groups (n)	Litters per group	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
Zenick et al. (1979)	Rat (CD)	750 mg/kg $Pb(Ac)_2$ (water)	Preconception to a) weaning (via dam) or b) termination (via dam and direct)	0-0 (?) Pb-0 (?) Pb-Pb (?)	5 5 5	?	42-? d	Operant (FI-1 min)	B.w. of Pb-Ss < 0-Ss from birth to weaning.	Pb-Pb group had sig. fewer rewarded responses across sessions than Pb-0 or 0-0 groups.

[a]Abbreviations and symbols:

?	information not given in report
ALA-D	delta aminolevulinic acid dehydrase
b.w.	body weight
C.	control group
CD	substrain of Sprague-Dawley
CRF	continuous reinforcement for each response
DRH	differential reinforcement of high response rates
DRL	differential reinforcement of low response rates
EXT	extinction
F_1	1st filial generation
F_2	2nd filial generation
F-344	Fischer-344
FI	fixed interval
FR	fixed ratio
i.p.	intraperitoneal injection
IRT	inter response time
L-E	Long-Evans
Pb	lead-exposed group (subscript indicates exposure level or other experimental condition)
$Pb(Ac)_2$	lead acetate
PbB	blood lead
$PbCO_3$	lead carbonate
PND	post-natal day
S	subject
s.c.	subcutaneous injection
S-D	Sprague Dawley
TEL	triethyl lead
TO	time out
U/l	µmole ALA/min x liter erythrocytes
VI	variable interval
W	Wistar
WGTA	Wisconsin general testing apparatus

[b]C_5-Ss came from litters of 5 pups each; C_{10}-Ss from litters of 10; both Pb groups from litters of 5 each.

[c]C_0-Ss sham injected; C_{15}-Ss injected with 15% ethanol.

[d]For Ss on zinc-replete diet; Ss on zinc-deficient diet had higher Pb concentrations.

[e]Weight-matched controls.

[f]Pair-fed and -watered controls.

[g]Inferred from information in report.

TABLE 12-5. RECENT ANIMAL TOXICOLOGY STUDIES OF LEAD'S EFFECTS ON LEARNING IN PRIMATES[a]

Reference	Species	Lead exposure		Treatment groups (n)	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
		conc. (medium)	period (route)						
Bushnell & Bowman (1979a) Expt. 1	*Macaca mulatta*	~0.53 or 1.15 mg/kg Pb (milk) adjusted to maintain target PbBs	Birth - 1 yr (direct)	C (4) Pb_1 (3) Pb_2 (3)	PbB (1st yr)[b]: C: ~5 μg/dl Pb_1: 37 Pb_2: 58	5-10 mo	WGTA (form discrim. reversal learning)	None	Both Pb groups retarded in reversal learning; Pb_2-Ss especially impaired on 1st reversal following overtraining.
Expt. 2 Test 1		~0.25 or 1.06 mg/kg Pb (milk) adjusted to maintain target PbBs	Birth - 1 yr (direct)	C (4) Pb_1 (4) Pb_2 (4)	PbB (1st yr)[b]: C: ~4 μg/dl Pb_1: 32 Pb_2: 65	1.5-4.5 mo	2-choice maze (discr. reversal learning) non-food reward	None	Pb_2-Ss sig. retarded on 1st reversal (confirms Expt. 1 using different task and reward to control for possible confounding by motivational or motor factors).
Test 2		--------------------Continuation of Expt. 2----------------				5-12 mo	WGTA (series of 4 reversal discr. problems)	None	Both Pb groups retarded in reversal learning; Pb_2-Ss impaired on 1st reversals regardless of prior overtraining.
Test 3		---------Continuation of Expt. 2--------- after exposure terminated at 12 mo			PbB (16 mo): C: ~5 μg/dl Pb_1: 19 Pb_2: 46	15 mo	WGTA (discr. reversal learning, more difficult cues)	None	Pb_2-Ss retarded on 1st reversal.

TABLE 12-5. (continued)

Reference	Species	Lead exposure conc. (medium)	 period (route)	Treatment groups (n)	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
Bushnell & Bowman (1979b)	*Macaca mulatta*	--Continuation of Bushnell & Bowman (1979a)--			PbB (56 mo): C: 4 µg/dl Pb_1: 5 Pb_2: 6	49-55 mo	WGTA (spatial discr. reversal learning)	None	Both Pb groups retarded in reversal learning; 3 Pb_2-Ss failed to retain motor pattern for operating WGTA from 2 yr earlier.
Mele et al. (1984)	*Macaca mulatta*	--Continuation of Bushnell & Bowman (1979a)--		C (4) Pb_1 (3) Pb_2 (3)	PbB (37 mo): C: 3 µg/dl Pb_1: 5 Pb_2: 11	33 mo	Operant (FI-1 min)	None	Rate of acceleration in FI pattern of responding sig. reduced in Pb_1 + Pb_2 Ss.
Levin and Bowman (1983)	*Macaca mulatta*	0.29 or 0.88 mg/kg Pb (milk)	Birth - 1 yr (direct)	C (3) Pb_1 (4) Pb_2 (3)	PbB (1st yr): C: ~5 µg/dl Pb_1: 40 Pb_2: 85	4-5 yr	WGTA-Hamilton search task (find food under 6 boxes)	None	Pb-Ss sig. slower to reach criterion (examine 6 boxes without repeats).
	Expt. 1 (Continuation of Expt. 2 of Bushnell & Bowman, 1979a)								
	Expt. 2 (Continuation of Expt. 4 of Bushnell & Bowman, 1979c)								
		3 or 6 mg/kg $Pb(Ac)_2$ (water)	Preconception - Birth (via mother)	C (5) Pb_1 (3) Pb_2 (4)	PbB (birth): C: 5 µg/dl Pb_1: 30 Pb_2: 55	4-5 yr	Same (find food under 8 boxes)	None	No sig. differences in Pb- and C-Ss; all Ss had equal difficulty with criterion of 8.
Laughlin et al. (1983)	*Macaca mulatta*	~10 mg/kg and/or ~0.5 mg/kg b.w. Pb (milk)	Weeks 5-6 and/or birth-1 yr (direct)	C (4) Pb_1 (4) Pb_2 (4) Pb_3 (4)	PbB (12 mo): C: 3.4 µg/dl Pb_1: 8.6 (pulse only) Pb_2 53.4 (chronic only) Pb_3 55.0 (chronic & pulse) (16 mo): C: 4 Pb_1: 7.8 Pb_2: 29.5 Pb_3: 30.2	a: 12 mo b: 16 mo	WGTA (discr. reversal learning: a: without overtrng; b: with overtrng.)	None	No differences between Pb- and C-Ss at 12 mo; Pb_1 and Pb_3 (pulse exposed) slower to reach criterion on reversal at 16 mo; no sig. difference between Pb_2- and C-Ss.

TABLE 12-5. (continued)

Reference	Species	Lead exposure		Treatment groups (n)	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
		conc. (medium)	period (route)						
Rice & Willes (1979)	*Macaca fascicularis*	500 μg/kg b.w. Pb (milk)	Birth - life (direct)	C (4) Pb (4)	PbB (200 d): C: <5 μg/dl Pb: 35-70 (400 d): Pb: 20-50	421-714 d	WGTA (form discrim. reversal)	None	Pb-Ss slower to learn successive reversals.
Rice et al. (1979)	------------Continuation of Rice & Willes (1979)----------				PbB (400+ d): 20-30 μg/dl	2.5-3 yr	Operant (multiple FI-TO)	None	Pb-Ss responded at higher rates, had shorter IRTs, and tended to respond more during time-out (unrewarded).
Rice (1984)	*Macaca fascicularis*	500 μg/kg b.w. Pb (milk)	Birth - life (direct)	C (4) Pb (4)	PbB (peak): C: <5 μg/dl Pb: 55.3 (steady state): Pb: 32.8	3-3.5 yr	Operant (delayed matching to sample for: 1) color 2) position)	None	Pb-Ss performed both tasks sig. worse than C-Ss, although no difference in acquisition or at 0 delay. Pb-Ss made perseverative errors on color matching task.
Rice (1985a)	*Macaca fascicularis*	50 or 100 μg/kg Pb (milk)	Birth - life (direct)	C (7) Pb_1 (8) Pb_2 (5)	PbB (peak): C: 3.5 μg/dl Pb_1: 15.4 Pb_2: 25.4 (steady state): C: 2.9 Pb_1: 10.9 Pb_2: 13.1	3-4 yr	Operant (1) form discr. reversal; 2) color discr. reversal; 3) form discr. reversal, color irrelevant)	None (incl. b.w. and blood chemistry)	Pb_1-Ss sig. worse than C-Ss on last 4 of 15 reversals for all 3 tasks, i.e., did not improve as much as C-Ss; Pb_2-Ss sig. worse than C-Ss on all reversals.

TABLE 12-5. (continued)

Reference	Species	Lead exposure conc. (medium)	Lead exposure period (route)	Treatment groups (n)	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
Rice (1985b)	*Macaca fascicularis*	2000 μg/kg b.w. Pb (milk)	Birth - life (direct)	C (6) Pb (6)	PbB (peak): C: 3.1 μg/dl Pb: 115.0 (steady state): C: 3.5 Pb: 33.0	a: 0-9 mo; b: 3-4 yr	Operant (a: FI 2 min or FR 10-40; b: mult FI-FR)	None	At 0-9 mo, Pb-Ss paused sig. longer; at 3-4 yr, Pb-Ss had sig. shorter IRTs, higher response rate and greater variability of response rate.
Rice and Gilbert (1985)	*Macaca fascicularis*	--------------------Continuation of Rice (1985a)--------------------					Operant (DRL)	None	Pb-Ss sig. worse in learning to respond at low rate; also sig. greater session-to-session variability in performance during terminal sessions.
Winneke et al. (1983); Lilienthal et al. (1983)	*Macaca mulatta*	350 or 600 ppm $Pb(Ac)_2$ (food)	Preconception -5 mo postnatal (via mother and direct)	C (6) Pb_1 (5) Pb_2 (6)	PbB (post-test): C: 9.6 μg/dl Pb_1: 51.7 Pb_2: 71.4	?	WGTA (series of 36 discrim. problems)	?	Sig. dose-related deficits in learning set formation in both Pb groups.
Zook et al. (1980)	*Macaca mulatta*	1) 300 mg/kg or 2) 100 mg/kg Pb (paint)	1) 10-23 wk from age 6-8 mo or 2) 43-113 d from age 5-12 d (direct)	C_1 $(3)^c$ Pb_1 (4) C_2 $(2)^c$ Pb_2 (4)	PbB (term.): C: 12 μg/dl Pb_1: 470 Pb_2: 96	1) 15-16 mo 2) 6 mo	WGTA (series of 10 stimulus discrim. problems)	Various clinical signs	No sig. difference in mean number of errors.

[a] Abbreviations:

b.w.	body weight
C	control group
DRL	differential reinforcement of low response rates
FI	fixed interval
FR	fixed ratio
IRT	inter response time
Pb	lead-exposed group (subscript indicates exposure level or other experimental condition)
$Pb(Ac)_2$	lead acetate
PbB	blood lead
S	subject
WGTA	Wisconsin general testing apparatus

[b] Corrected annual averages obtained from Bushell (1978).

[c] age-matched controls.

selected for the great majority of the behavioral studies, despite concerns that have repeatedly been expressed concerning the appropriateness of this species as a subject for behavioral investigation (e.g., Lockard, 1968, 1971; Zeigler, 1973).

A number of studies have reported alterations in learning task performances by rats with blood lead levels below 30 µg/dl. The lowest exposure level to be significantly associated with a behavioral effect was reported by Bushnell and Levin (1983), who exposed rats from PND 21 (postweaning) to a drinking water solution of 10 ppm lead for 35 days. Although blood lead concentrations were not measured, brain lead levels at PND 57 (the day following termination of lead exposure) averaged 0.05 µg/g. By comparison with other studies in which lead levels in blood as well as brain were determined at a similar age (Collins et al., 1984; Grant et al., 1980; Bull et al., 1979), it would appear that the animals in question probably had maximum blood lead levels under 20 µg/dl.

The behavior assessed by Bushnell and Levin (1983)--spontaneous alternation in a radial arm maze--could be described as a form of natural or unrewarded learning, since there was no experimenter-imposed contingency of reinforcement for alternating between different arms of the maze before reentering a previously selected arm. Other testing paradigms have also revealed behavioral alterations in subjects exposed to quite low levels of lead. For example, Cory-Slechta et al. (1985) reported significant effects in rats exposed postweaning to a 25-ppm lead acetate solution for their drinking water. Exposure continued throughout the course of the experiment, with blood lead levels stabilizing at 15-20 µg/dl by PND 99 (the first point of measurement), by which time the behavioral effects were already evident. In this case, the outcome was a significantly higher response rate in the lead-exposed animals on a fixed-interval operant schedule of food reinforcement. Consistent with this finding, the interval between bar-press responses was also significantly shorter in the lead-exposed rats. Cory-Slechta and her colleagues obtained similar results at higher exposure levels in a series of earlier studies (Cory-Slechta and Thompson, 1979; Cory-Slechta et al., 1981, 1983), even when the operant schedule or contingency for reinforcement was rather different. For example, in the experiment by Cory-Slechta et al. (1981), a bar-press of a certain minimum duration was required before the rats could be rewarded. Subjects exposed to 100 or 300 ppm lead acetate solutions for drinking water were impaired in their ability to meet this response requirement.

A tendency to respond more rapidly (higher response rate, shorter inter-response times, shorter response latencies) or to respond even when inappropriate (when no reward is provided for responses or when reward is specifically withheld for responding) has been reported in quite a few other studies of lead-exposed rats (Alfano and Petit, 1985; Angell and Weiss, 1982; Cory-Slechta and Thompson, 1979; Cory-Slechta et al., 1983; Dietz et al., 1978; Gross-Selbeck and Gross Selbeck, 1981; Hastings et al., 1984; Nation et al., 1982; Overmann, 1977; Padich and Zenick, 1977; Rosen et al., 1985; Taylor et al., 1982; Winneke et al., 1982b; Zenick et al., 1978). In many of these investigations the lead exposure levels were rather low, resulting in blood lead concentrations under 30 µg/dl at the time of assessment (although peak levels may have been considerably higher).

Additional forms of impairment have been reported in studies using other behavioral testing paradigms. Winneke and his associates (Winneke et al., 1977, 1982b; Schlipköter and Winneke, 1980) employed an apparatus requiring the subjects to discriminate between stimuli of different sizes and found that lead-exposed rats were slower to learn the discrimination or tended to repeat errors more than control subjects. In these studies, exposure occurred *in utero* as well as via the dam's milk and directly through the subjects' drinking water post-weaning. Blood lead levels around PND 16 were less than 30 µg/dl in the study of Winneke et al. (1977). A number of other reports have also noted impaired discrimination acquisition or performance in various testing paradigms with rats (Booze et al., 1983; Geist and Mattes, 1979; Hastings et al., 1979; Kowalski et al., 1982; McLean et al., 1982; Overmann, 1977; Penzien et al., 1982; Zenick et al., 1978).

Nonhuman primates have been studied in several studies of the effects of lead on learning ability (Table 12-5). For the most part, these studies have exposed monkeys directly to lead from birth and then analyzed the subjects' ability to discriminate stimuli differentially associated with rewards. A number of these studies were conducted by Bowman and his colleagues (Bushnell and Bowman, 1979a,b; Levin and Bowman, 1983; Laughlin et al., 1983; Mele et al., 1984). Using a variety of tasks and different groups of subjects (as well as the same subjects followed for several months or years after exposure terminated), these investigators have consistently found evidence of impaired learning ability in monkeys, even after the subjects' blood lead levels had dropped to control values, i.e., ~5 µg/dl (see Section 12.4.3.1.5 for further discussion on the persistence of neonatal exposure effects). One type of test that has been frequently used to detect lead-induced impairment in primates is the discrimination reversal task. Discrimination reversal tasks require the subject to correctly respond to one of two stimuli associated with reward and then, once that task has been mastered, to make the reverse discrimination, i.e., respond only to the cue formerly unpaired with reward. Greater difficulty in learning such reversals by lead-exposed monkeys has been shown repeatedly by Bowman and his colleagues.

The above findings have been generally confirmed and extended by Rice and her colleagues (Rice, 1984, 1985a,b; Rice and Willes, 1979; Rice and Gilbert, 1985; Rice et al., 1979). Although Rice's studies used operant conditioning tasks to a greater extent than Bowman's studies, impaired learning ability was consistently demonstrated, even in some cases where the monkeys' peak blood lead levels reached only 15 µg/dl and steady state levels were only 11 µg/dl. Rice (1985a) particularly noted the consistency of her results with Bushnell and

Bowman's (1979a,b) finding of impaired ability to learn discrimination reversal tasks. Similar results were also obtained with rats by Driscoll and Stegner (1976), but not by Hastings et al. (1984) or Rabe et al. (1985). In addition, a relatively high degree of response variability was found in Rice's lead-treated monkeys as was found in lead-treated rats (Cory-Slechta et al., 1985; Cory-Slechta et al., 1981, 1983; Cory-Slechta and Thompson, 1979; Dietz et al., 1978).

Another finding from Rice's studies that is consistent with the results of other studies is the tendency of lead-treated subjects to respond excessively or inappropriately. For example, lead-exposed monkeys tended to respond more than control subjects during "time-outs" in operant schedules when responses were unrewarded (Rice and Willes, 1979). They also tended to have higher response rates and shorter interresponse times on fixed-interval operant schedules (Rice, 1985b). Where the schedule of reinforcement required a low rate of responding before reward could be delivered, the lead-treated subjects were significantly slower than controls to learn the appropriate pattern of responding (Rice and Gilbert, 1985). Such subjects also made more perseverative errors on operant "matching-to-sample" tasks that required them to direct their responses according to stimulus colors (Rice, 1984).

These findings bear striking resemblence to the results of several studies of lead-exposed rats which, as mentioned above, tended to respond excessively or more rapidly than controls or than conditions of the experiment would have otherwise produced. Such tendencies have been characterized as "hyper-reactivity" by some investigators (e.g., Winneke et al., 1982b). However, this concept (not to be confused with hyperactivity per se) is only descriptive, not explanatory. Speculation about the neural mechanisms responsible for such behavior has tended to focus on the hippocampus, because of the behavioral similarities with animals having experimental lesions of the hippocampus (Petit and Alfano, 1979; Petit et al., 1983) (see also Sections 12.4.3.2.1 and 12.4.3.5). It should be noted that, at sufficiently high exposure levels, increased response tendencies give way to decreased responding (e.g., Cory-Slechta and Thompson, 1979; Angell and Weiss, 1982). Cory-Slechta et al. (1983) have argued that this curvilinear dose-response relationship may be due at least in part to differences in the time required for response rates to reach their maximum as a function of different exposure levels. In their study, rats exposed to higher concentrations of lead took longer to reach their peak response rate; consequently, assessing performance earlier would make the responding of the higher lead exposure group appear depressed, while responding of a lower exposure group would appear to be elevated relative to controls (Cory-Slechta et al., 1983). Of course, at sufficiently toxic doses, responding obviously declines if the subjects are no longer able to perform the necessary motor responses.

It seems clear from the above studies that alterations in the behavior of rats and monkeys occur as a consequence of chronic exposure to relatively low levels of dietary lead. In a number of instances (e.g., Mele et al., 1984; Bushnell and Bowman, 1979b) these perturbations were evident even after blood lead concentrations had returned to nearly normal levels, although earlier exposure had probably been much higher. One study reported learning disturbances in monkeys whose average steady-state blood lead level was around 11 µg/dl and whose peak level reached only about 15 µg/dl (Rice, 1985a). A number of studies with rats found evidence of behavioral deficits at blood lead levels below 30 µg/dl, and in at least one case the blood lead level probably did not exceed 20 µg/dl.

12.4.3.1.4 Effects of lead on social behavior. The social behavior and organization of even phylogenetically closely related species may be widely divergent. For this and other reasons, there is little or no basis to assume that, for example, aggressiveness in a lead-treated rhesus monkey provides a model of aggressiveness in a lead-exposed human child. However, there are other compelling grounds for including animal social behavior in the present review. As in the case of nonsocial behavior patterns, characteristics of an animal's interactions with conspecifics may reflect neurological (especially CNS) impairment due to toxic exposure. Also, certain aspects of animal social behavior have evolved for the very purpose (in a non-teleological sense) of indicating an individual's physiological state or condition (Davis, 1982). Such behavior could potentially provide a sensitive and convenient indicator of toxicological impairment.

Two early reports (Silbergeld and Goldberg, 1973; Sauerhoff and Michaelson, 1973) suggested that lead exposure produced increased aggressiveness in rodents. Neither report, however, attempted to quantify these observations of increased aggression. Later, Hastings et al. (1977) examined aggressive behavior in rats that had been exposed to lead via their dams' milk. Solutions containing 0, 0.01, or 0.05 percent lead as lead acetate constituted the dams' drinking water from parturition to weaning at PND 21, at which time exposure was terminated. This lead treatment produced no change in growth of the pups. Individual pairs of male offspring (from the same treatment groups) were tested at PND 60 for shock-elicited aggression. Both lead-exposed groups (average blood lead levels of 5 and 9 µg/dl and brain lead levels of 8 and 14 µg/100g) showed significantly less aggressive behavior than the control group. There were no significant differences among the groups in the flinch/jump thresholds for shock, which suggests that the differences seen in shock-elicited aggression were not caused by differences in sensitivity to shock.

A study by Drew et al. (1979) utilized apomorphine to induce aggressive behavior in 90-day-old rats and found that earlier lead exposure attenuated the drug-induced aggressiveness.

Lead exposure occurred between birth and weaning primarily through the dams' milk or through food containing 0.05 percent lead as lead acetate. No blood or tissue concentrations of lead were measured. There were no significant differences in the weights of the lead-treated and control animals at PND 10, 20, 30, or 90.

Using laboratory mice exposed as adults, Ogilvie and Martin (1982) also observed reduced levels of aggressive behavior. Since the same subjects showed no differences in vitality or open field activity measures, the reduction in aggressiveness did not appear to be due to a general effect of lead on motor activity. Blood lead levels were estimated from similarly treated groups to be approximately 160 μg/dl after 2 weeks of exposure and 101 μg/dl after 4 weeks of exposure.

Cutler (1977) used ethological methods to assess the effects of lead exposure on social behavior in laboratory mice. Subjects were exposed from birth (via their dams' milk) and post-weaning to a 0.05 percent solution of lead as lead acetate (average brain lead concentrations were 2.45 nmol/g for controls and 4.38 nmol/g for experimental subjects). At 8 weeks of age social encounters between subjects from the same treatment group were analyzed in terms of a number of specified, identifiable behavioral and postural elements. The frequency and duration of certain social and sexual investigative behavior patterns were significantly lower in lead-treated mice of both sexes than in controls. Lead-exposed males also showed significantly reduced agonistic behavior compared with controls. Overall activity levels (nonsocial as well as social behavior) were not affected by the lead treatment. Average body weights did not differ for the experimental and control subjects at weaning or at the time of testing.

A more recent study by Cutler and coworkers (Donald et al., 1981) used a similar paradigm of exposure and behavioral evaluation, except that exposure occurred either only prenatally or postnatally and testing occurred at two times, 3-4 and 14-16 weeks of age. Statistically significant effects were found only for the postnatal exposure group. Although total activity in postnatally exposed mice did not differ from that of controls at either age of testing, the incidence of various social activities did differ significantly. As juveniles (3-4 weeks old), lead-treated males (and to some extent, females) showed decreased social investigation of a same-sex conspecific. This finding seems to be consistent with Cutler's (1977) earlier observations made at 8 weeks of age. Aggressive behavior, however, was almost nonexistent in both control and lead-treated subjects in the later study, and so could not be compared meaningfully. Although the authors do not comment on this aspect of their study, it seems likely that differences in the strains of laboratory mice used as subjects could well have been responsible for the lack of aggressive behavior in the Donald et al. (1981) study (see, e.g., Adams and Boice, 1981). Later testing at 14-16 weeks revealed that lead-exposed female subjects engaged in significantly more investigative behavior of a social or sexual nature than did control subjects, while males still showed significant reductions in such behavior when encountering another mouse of the same sex. This apparent disparity between male and female mice is one of relatively few reports of gender differences in sensitivity to lead's effects on the nervous system (cf. Cutler, 1977; Verlangieri, 1979). In this case, Donald et al. (1981) hypothesized that the disparity might have been due to differences in brain lead concentrations: 74.7 μmol/kg in males versus 191.6 μmol/kg in females (blood lead concentrations were not measured).

The social behavior of rhesus monkeys has also been evaluated as a function of early lead exposure. A study by Allen et al. (1974) reported persistent perturbations in various aspects of the social behavior of lead-exposed infant and juvenile monkeys, including increased clinging, reduced social interaction, and increased vocalization. However, exposure conditions varied considerably in the course of this study, with overt toxicity being evident as blood lead levels at times ranged higher than 500 μg/dl.

A more recent study consisting of four experiments (Bushnell and Bowman, 1979c) also examined social behavior in infant rhesus monkeys, but under more systematically varied exposure conditions. In experiments 1 and 2, daily ingestion of lead acetate during the first year of life resulted in blood lead levels of 30-100 μg/dl, with consequent suppression of play activity, increased clinging, and greater disruption of social behavior when the play environment was altered. Experiment 3, a comparison of chronic and acute lead exposure (the latter resulting in a peak blood lead concentration of 250-300 μg/dl during weeks 6-7 of life), revealed little effect of acute exposure except in the disruption that occurred when the play environment was altered. Otherwise, only the chronically exposed subjects differed significantly from controls in various categories of social behavior. Experiment 4 of the study showed that prenatal exposure alone, with blood lead concentrations of exposed infants ranging between 33 and 98 μg/dl at birth, produced no detectable behavioral effects under the same procedures of evaluation. Overall, neither aggressiveness nor dominance was clearly affected by lead exposure.

Another aspect of social behavior--interaction between mothers and their offspring--was examined in lead-exposed rats by Zenick et al. (1979). Dams chronically received up to 400 mg/kg lead acetate in their drinking water on a restricted daily schedule (blood lead concentrations averaged 96.14 ± 16.54 μg/dl in the high-exposure group at day 1 of gestation). Dams and their litters were videotaped on PND 1-11, and the occurrence of certain behavior patterns (e.g., lying with majority of pups, lying away from pups, feeding) was tabulated by the experimenters. In addition, dams were tested for their propensity to retrieve pups removed from the nest. Neither analysis revealed significant effects of lead exposure on the behavior of the dams. However, restricted access to drinking water (whether lead-treated or not) appeared to confound the measures of maternal behavior.

A more recent investigation of maternal behavior and offspring development in rats exposed via their food revealed significant lead-related alterations in the behavioral interactions between pups and their dams (Barrett and Livesey, 1983). Pups whose blood lead levels ranged from 20 to 60 µg/dl at weaning were slower to leave the nest area to find the dam for suckling or to climb into food hoppers for solid food. The lead-exposed dams, with blood lead values of 30-60 µg/dl at weaning, in turn spent more time in the nest than control dams. These findings are consistent with other observations of retarded pup development and increased retrieval of pups to the nest by dams exposed to low levels of lead (Davis, 1982). As Barrett and Livesey (1983) note, the net effect of this altered motor-infant interaction is difficult to predict. While extra maternal care could help compensate for slowed development caused by lead, it could also exacerbate the situation by depriving the pups of the outside stimulation needed for normal development (Levitsky et al., 1975).

The above studies suggest that animal social behavior or behavioral interactions may be altered in various ways by exposure to lead. Aggressive behavior in particular is, if anything, reduced in laboratory animals as a result of exposure to lead. Certain other aspects of social behavior in laboratory mice, namely components of sexual interaction and social investigation, also appear to be reduced in lead-treated subjects, although there may be gender differences in this regard following chronic post-maturational exposure. In additon, young rhesus monkeys appear to be sensitive to the disruptive effects of lead on various aspects of social behavior. These alterations in social behavior in several mammalian species are indicative of altered neural functioning as a consequence of lead exposure.

12.4.3.1.5 Persistence of neonatal exposure effects. The specific question of persisting, long-term consequences of lead exposure on the developing organism has been addressed in a number of studies by carrying out behavioral testing some time after the termination of lead exposure. For example, such evidence of long-term effects has been reported for rhesus monkeys by Bushnell and Bowman (1979b). Their subjects were fed lead acetate so as to maintain blood lead levels of either 50 ± 10 (low-lead) or 80 ± 10 µg/dl (high-lead) throughout the first year of life (actual means and standard errors for the year were reported as 31.71 ± 2.75 and 65.17 ± 6.28 µg/dl). Lead treatment was terminated at 12 months of age, after which blood lead levels declined to around 5-6 µg/dl at 56 months. At 49 months of age the subjects were re-introduced to a discrimination reversal training procedure using new discriminative stimuli. Despite their extensive experience with the apparatus (Wisconsin General Test Apparatus) during the first two years of life, most of the high-lead subjects failed to retain the simple motor pattern (pushing aside a small wooden block) required to operate the apparatus. Remedial training largely corrected this deficit. However, both high- and low-lead groups required significantly more trials than the control group ($p < 0.05$) to reach criterion performance levels. This difference was found only on the first discrimination task and nine reversals of it. Successive discrimination problems showed no differential performance effects, which indicates that with continued training the lead-treated subjects were able to achieve the same level of performance as controls.

Other studies with monkeys have also shown behavioral alterations some time after blood lead concentrations have returned to essentially normal levels (Laughlin et al., 1983; Levin and Bowman, 1983; Mele et al., 1984). Some evidence suggests that rats may show similar effects (e.g., Angell and Weiss, 1982; Gross-Selbeck and Gross-Selbeck, 1981), but other evidence implies that behavioral effects eventually disappear after lead exposure ends (e.g., Flynn et al., 1979; Hastings et al., 1977, 1984; Padich and Zenick, 1977; Rosen et al., 1985; Schlipköter and Winneke, 1980). Even if some behavioral changes are reversible, it does not follow, of course, that all behavioral effects of early lead exposure are reversible. Most likely, neurotoxic outcomes differ in their persistence, and these differences account for any apparent inconsistency in the above findings.

12.4.3.2 Morphological Effects

12.4.3.2.1 In vivo studies. Recent key findings on the morphological effects of *in vivo* lead exposure on the nervous system are summarized in Table 12-6.* It would appear that certain types of of glial cells are sensitive to lead exposure, as Reyners et al. (1979) found a decreased density of oligodendrocytes in cerebral cortex of young rats exposed from birth to 0.1 percent lead in their food. Exposures to higher concentrations (0.2-0.4 percent lead salts), especially if begun during the prenatal period (Bull et al., 1983), can reduce synaptogenesis and retard dendritic development in the cerebral cortex (McCauley and Bull, 1978; McCauley et al., 1979, 1982) and the hippocampus of developing rats (Campbell et al., 1982; Alfano and Petit, 1982). Some of these effects, e.g., those on the hippocampus, appear to be transient (Campbell et al., 1982) and may be related to lead-induced alterations in size and/or bioavailability of sub-cellular zinc pools (Sato et al., 1984). Interestingly, an apparent compensatory hypertrophy of both neurons and neuropil appears in certain areas of the hippocampus of 90-day old rats who were exposed perinatally to lead (Kawamoto et al., 1984).

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*Concentrations of lead reported in the following sections are given as percent lead salt. For comparison with exposure concentrations discussed in other sections of this document, multiply by 10,000 to obtain value in parts per million (ppm). Example: 1% = 10,000 ppm.

TABLE 12-6. SUMMARY OF KEY STUDIES OF MORPHOLOGICAL EFFECTS OF IN VIVO LEAD EXPOSURE*

Species	Exposure protocol	Peak blood lead level	Observed effect	Reference
Young rats	0.1% Pb^{2+} in chow PND 0-90		Decreased density of oligodendrocytes in cerebral cortex	Reyners et al. (1979)
	0.1% $Pb(Ac)_2$ in dams' drinking water PND 0-60		Focal necrosis of photoreceptor cells and cells in inner nuclear layer of retina	Santos-Anderson et al. (1984)
	0.1% $Pb(Ac)_2$ in dams' drinking water PND 0-32		Significant inhibition in myelin deposition and maturation in whole brain	Stephens and Gerber (1981)
	0.2% $PbCl_2$ in dams' drinking water from gestation thru PND 20	80 µg/dl (at birth)	Less mature synaptic profile in cerebral cortex at PND 15	McCauley and Bull (1978); McCauley et al. (1979)
			30% reduction in synaptic density in cerebral cortex at PND 15 (returned to normal at PND 21)	McCauley et al. (1982)
	0.2% $Pb(Ac)_2$ in dams' drinking water PND 0-25		15-30% reduction in synaptic profiles in hippocampus	Campbell et al. (1982)
	0.4% $PbCO_3$ in dams' drinking water PND 0-30	300-400 µg/dl (PND 26)	Retardation in temporal sequence of hippocampal dendritic development	Alfano and Petit (1982); Petit et al. (1983)
	0.5% $Pb(Ac)_2$ in dams' drinking water PND 0-21		10-15% reduction in number of axons in optic nerve; skewing of fiber diameters to smaller sizes	Tennekoon et al. (1979)
	1% $PbCO_3$ in chow PND 0-60	385 µg/dl (PND 21)	Retardation of cortical synaptogenesis over and above any nutritional effects	Averill and Needleman (1980)
	4% $PbCO_3$ in dams' chow PND 0-28	258 µg/dl (PND 28)	13% reduction in cortical thickness and total brain weight; reduction in synaptic density	Petit and LeBoutillier (1979)
	4% $PbCO_3$ in dams' chow PND 0-25		Reduction in hippocampal length and width; similar reduction in afferent projection to hippocampus	Alfano et al. (1982)
Adult rats	4% $PbCO_3$ in chow for 3 mos.		Delay in onset and peak of Schwann cell division and axonal regrowth in regenerating nerves	Ohnishi and Dyck (1981)
	4% $PbCO_3$ in chow PND 0-150	300 µg/dl (PND 150)	Demyelination of peripheral nerves beginning PND 20-35	Windebank et al. (1980)

*Abbreviations:

PND:	postnatal day
$Pb(Ac)_2$:	lead acetate
$PbCO_3$:	lead carbonate

Suckling rats subjected to increasing exposures of lead exhibit more pronounced effects, such as reduction in the number and average diameter of axons in the optic nerve at 0.5 percent lead acetate exposure (Tennekoon et al., 1979), a general retardation of cortical synaptogenesis at 1.0 percent lead carbonate exposure (Averill and Needleman, 1980), or a reduction in cortical thickness at 4.0 percent lead carbonate exposure (Petit and LeBoutillier, 1979). This latter exposure concentration also causes a delay in the onset and peak of Schwann cell division and axonal regrowth in regenerating peripheral nerves in chronically exposed adult rats (Ohnishi and Dyck, 1981). In short, both neuronal and glial components of the nervous system appear to be affected by neonatal or chronic lead exposure.

Organolead compounds have also been demonstrated to have a deleterious effect on the morphological development of the nervous system. Seawright et al. (1980) administered triethyl lead acetate (Et_3Pb) by gavage to weanling (40-50 g) and "young adult" (120-150 g) rats. Single doses of 20 mg Et_3Pb/kg caused impaired balance, convulsions, paralysis, and coma in both groups of treated animals. Peak levels in blood and brain were noted two days after exposure, with extensive neuronal necrosis evident in several brain regions by three days post-treatment. Weekly exposures to 10 mg Et_3Pb/kg for 19 weeks resulted in less severe overt signs of intoxication (from which the animals recovered) and moderate to severe loss of neurons in the hippocampal region only.

12.4.3.2.2 In vitro studies. Björklund et al. (1980) placed tissue grafts of developing nervous tissue in the anterior eye chambers of adult rats. When the host animals were given 1 or 2 percent lead acetate in their drinking water, the growths of substantia nigral and hippocampal, but not cerebellar, grafts were retarded. Grafts of the developing cerebral cortex in host animals receiving 2 percent lead exhibited a permanent 50 percent reduction in size (volume), whereas 1 percent lead produced a slight increase in size in this tissue type. The authors felt that this anomalous result might be explained by a hyperplasia of one particular cell type at lower concentrations of lead exposure.

Organolead compounds have also been demonstrated to affect neuronal growth (Grundt et al., 1981). Cultured cells from embryonic chick brain were exposed to 3.16 μM triethyllead chloride in the incubation medium for 48 hr, resulting in a 50 percent reduction in the number of cells exhibiting processes. There was no observed effect on glial morphology.

Other investigations have focused on morphological aspects of the blood-brain barrier and its possible disruption by lead intoxication (Kolber et al., 1980). Capillary endothelial cells isolated from rat cerebral cortex and exposed to 100 μM lead acetate in vitro (Silbergeld et al., 1980b) were examined by electron microscopy and X-ray microprobe analysis. Lead deposits were found to be sequestered preferentially in the mitochondria of these cells in much the same manner as calcium. This affinity may be the basis for lead-induced disruption of transepithelial transport of Ca^{2+} and other ions.

12.4.3.3 *Electrophysiological Effects*.

12.4.3.3.1 *In vivo studies*. Recent key findings on the electrophysiological effects of *in vivo* lead exposure are summarized below in Table 12-7. The visual system appears to be particularly susceptible to perturbation by neonatal lead exposure. Suckling rats whose dams were given drinking water containing 0.2 percent lead acetate had significant alterations in their visual evoked responses (VERs) and decreased visual acuity at PND 21, at which time their blood lead levels were 65 µg/dl (Cooper et al., 1980; Fox et al., 1977; Impelman et al., 1982; Fox and Wright, 1982; Winneke, 1980). Both of these observations are indicative of depressed conduction velocities in the visual pathways. These same exposure levels also increased the severity of the maximal electroshock seizure (MES) response in weanling rats who exhibited blood lead levels of 90 µg/dl (Fox et al., 1978, 1979). The authors speculated that neonatal lead exposure acts to increase the ratio of excitatory to inhibitory systems in the developing cerebrospinal axis. Such exposure can also lead to lasting effects on the adult nervous system, as indicated by persistent decreases in visual acuity and spatial resolution in 90-day old rats exposed only from birth to weaning to 0.2 percent lead acetate (Fox et al., 1982). A 38-percent decrease in the number of cholinergic receptors in the visual cortex of adult rats treated in this manner (Costa and Fox, 1983) may represent the morphological basis for this finding.

The adult nervous system is also vulnerable to lead-induced perturbation at low levels of exposure. For example, Hietanen et al. (1980) found that chronic exposure of adult rabbits to 0.2 percent lead acetate in drinking water resulted in an 85 percent inhibition of motor conduction velocity in the sciatic nerve; adult rabbits fed 165 mg lead carbonate per day for 5 days (Kim et al., 1980) showed a 75 percent increase in Ca^{2+} retention time in incubated brain slices, indicating that lead inhibits the mediated efflux of Ca^{2+}.

12.4.3.3.2 *In vitro studies*. Palmer et al. (1981) and Olson et al. (1981) looked at intraocular grafts of cerebellar tissue from 14- to 15-day-old rats in host animals treated for 2 months with drinking water containing 1 percent lead acetate, followed by plain water for 4-5 months. They found no alterations in total growth or morphology of cerebellar grafts in treated versus control hosts, yet the Purkinje neurons in the lead-exposed grafts had almost no spontaneous activity. Host cerebellar neurons, on the other hand, and both host and graft neurons in control animals, all exhibited significant levels of spontaneous activity. It should be noted that when these investigators looked at the effects of lead on intraocular grafts of other areas of fetal rat brain, i.e., substantia nigra, hippocampus, and parietal cortex, they found significant delays in the growth of these grafts (Olson et al., 1984). Furthermore, attempts by this group to replicate their findings *in vivo* by using neonatal rats exposed from gestation to PND 20 to 0.5 percent lead acetate in drinking water have been unsuccessful (Palmer et al., 1984).

TABLE 12-7. SUMMARY OF KEY STUDIES OF ELECTROPHYSIOLOGICAL EFFECTS OF IN VIVO LEAD EXPOSURE*

Species	Exposure protocol	Peak blood lead level	Observed effect	Reference
Suckling rat	0.2% $Pb(Ac)_2$ in dams' drinking water PND 0-20	90 µg/dl (PND 20)	More rapid appearance and increased severity of MES response	Fox et al. (1978, 1979)
	0.2% $Pb(Ac)_2$ in dams' drinking water PND 0-21	65 µg/dl (PND 21)	1) Increased latencies and decreased amplitudes of primary and secondary components of VER; 2) decreased conduction velocities in visual pathways; 3) 25-50% decrease in scotopic visual acuity 4) persistent decreases in visual acuity and spatial resolution at PND 90	Fox et al. (1977); Impelman et al. (1982); Cooper et al. (1980); Winneke (1980); Fox and Wright (1982); Fox et al. (1982)
Young rhesus monkeys	$Pb(Ac)_2$ solutions in food PND 0-365	300 µg/dl (PND 60) 85 µg/dl	Severe impairment of discrimination accuracy; loss of scotopic function	Bushnell et al. (1977)
Adult rabbit	0.2% $Pb(Ac)_2$ in drinking water for 4 weeks		85% reduction in motor conduction velocity of sciatic nerve	Hietanen et al. (1980)

*Abbreviations:

PND: postnatal day

$Pb(Ac)_2$: lead acetate

MES: maximal electroshock seizure

VER: visual evoked response

Taylor et al. (1978) recorded extracellularly from cerebellar Purkinje cells in adult rats both in situ and in intraocular grafts in an effort to determine what effect lead had on the norepinephrine (NE)-induced inhibition of Purkinje cell spontaneous discharge. Application of exogenous NE to both in situ and in oculo cerebellum produced 61 and 49 percent inhibitions of spontaneous activity, respectively. The presence of 5-10 μM lead reduced this inhibition to 28 and 13 percent, respectively. This "disinhibition" was specific for NE, as responses to both cholinergic and parallel fiber stimulation in the same tissue remained the same. Furthermore, application of lead itself did not affect spontaneous activity, but did inhibit adenylate cyclase activity in cerebellar homogenates at the same concentration required to disinhibit the NE-induced reduction of spontaneous activity (3-5 μM).

Fox and Sillman (1979) and Sillman et al. (1982) looked at receptor potentials in the isolated, perfused bullfrog retina and found that additions of lead chloride caused a reversible, concentration-dependent depression of rod (but not cone) receptor potentials. Concentrations as low as 1 μM produced an average 5 percent depression, while 25-60 μM produced an average 34 percent depression.

Evidence that lead does indeed resemble other divalent cations, in that it appears to interfere with chemically-mediated synaptic transmission, has also been obtained in studies of peripheral nerve function. For example, lead is capable of blocking neural transmission at peripheral adrenergic synapses (Cooper and Steinberg, 1977). Measurements of the contraction force of the rabbit saphenous artery following stimulation of the sympathetic nerve endings indicated that lead blocks muscle contraction by an effect on the nerve terminals rather than by an effect on the muscle. Since the response recovered when the Ca^{2+} concentration was increased in the bathing solution, it was concluded that lead does not deplete transmitter stores in the nerve terminals, but more likely blocks NE release (Cooper and Steinberg, 1977; Pickett and Bornstein, 1984; Kober and Cooper, 1976).

It has also been demonstrated that lead depresses synaptic transmission at the peripheral neuromuscular junction by impairing acetylcholine (ACh) release from presynaptic terminals (Kostial and Vouk, 1957; Manalis and Cooper, 1973; Cooper and Manalis, 1974). This depression of neurotransmitter release evoked by nerve stimulation is accompanied by an increase in the spontaneous release of ACh, as evidenced by the increased frequency of spontaneous miniature endplate potentials (MEPPs) (Atchison and Narahashi, 1984; Kolton and Yaari (1982) and Manalis et al. (1984) found that this increase in MEPPs in the frog nerve/muscle preparation could be induced by lead concentrations as low as 5 μM and is probably due to competitive inhibition of Ca^{2+} binding (Cooper et al., 1984).

The effects of lead on neurotransmission within the central nervous system have also been studied. For example, investigation of the in vitro effects of lead on Ca^{2+} binding on caudate synaptosomes was carried out by Silbergeld and Adler (1978) . They determined that 50 μM lead caused an 8-fold increase in $^{45}Ca^{2+}$ binding and that in both control and lead-treated preparations the addition of ATP increased binding, while ruthenium red and Ca^{2+} decreased it. Further findings in this series of experiments demonstrated that lead inhibits the Na^{+}-stimulated loss of Ca^{2+} by mitochondria and that blockade of dopamine (DA) uptake by 5 μM benztropine reversed the lead-stimulated increase in Ca^{2+} uptake by synaptosomes. The authors concluded that lead affects the normal mechanisms of Ca^{2+} binding and uptake, perhaps by chelating with DA in order to enter the nerve terminal. By inhibiting the release of Ca^{2+} bound to mitochondria there, lead essentially causes an increase in the Ca^{2+} concentration gradient across the nerve terminal membrane. As a result, more Ca^{2+} would be expected to enter the nerve terminal during depolarization, thus effectively increasing synaptic neurotransmission at dopaminergic terminals without altering neuronal firing rates.

12.4.3.4 Biochemical Alterations. The majority of previous investigations of biochemical alterations in the nervous system following exposure to lead have focused on perturbations of various neurotransmitter systems, probably because of the documentation extant on the neurophysiological and behavioral roles played by these transmitters. Recently, however, somewhat more attention has been centered on the impact of lead exposure on energy metabolism and other cellular homeostatic mechanisms such as protein synthesis and glucose transport. A significant portion of this work has, however, been conducted *in vitro*.

12.4.3.4.1 In vivo studies. Recent key findings on the biochemical effects of *in vivo* exposure are summarized in Table 12-8. Although the majority of recent work has continued to focus on neurotransmitter function, it appears that the mechanisms of energy metabolism are also particularly vulnerable to perturbation by lead exposure. McCauley, Bull, and coworkers have demonstrated that exposure of prenatal rats to 0.02 percent lead chloride in their dams' drinking water leads to a marked reduction in cytochrome content in cerebral cortex, as well as a possible uncoupling of energy metabolism. Although the reduction in cytochrome content is transient and disappears by PND 30, it occurs at blood lead levels as low as 36 μg/dl (McCauley and Bull, 1978; Bull et al., 1979); delays in the development of energy metabolism may be seen as late as PND 50 (Bull, 1983). [See Section 12.2.1.3 for a discussion of lead effects on mitochondrial function.]

There does not appear to be a selective vulnerability of any particular neurotransmitter system to the effects of lead exposure. Pathways utilizing dopamine (DA), norepinephrine (NE), serotonin (5-HT), γ-aminobutyric acid (GABA), and acetylcholine (ACh) as neurotransmitters are all reported to be affected in neonatal animals at lead-exposure concentrations of 0.2-2.0 percent lead salts in dams' drinking water (see Shellenberger, 1984 for an exhaustive review of this literature). Although the blood lead values reported following exposure to the lower lead concentrations (0.2-0.25 percent lead acetate or lead chloride)

TABLE 12-8. SUMMARY OF KEY STUDIES ON BIOCHEMICAL EFFECTS OF IN VIVO LEAD EXPOSURE

Subject	Exposure protocol	Peak blood lead level	Observed effect	Reference
Suckling rat	0.004% $Pb(Ac)_2$ in dams' drinking water PND 0-35	-	Decline in synthesis and turnover of striatal DA	Govoni et al. (1979, 1980); Memo et al. (1980a, 1981)
	0.02% $PbCl_2$ in dams' drinking water from gestation thru PND 21	80 μg/dl (at birth) 36 μg/dl (PND 21)	1) Transient 30% reduction in cytochrome content of cerebral cortex; 2) possible uncoupling of energy metabolism 3) delays in development of energy metabolism	McCauley and Bull (1978); McCauley et al. (1979); Bull et al. (1979); Bull (1983)
	0.2% $Pb(Ac)_2$ in dams' drinking water PND 0-21	47 μg/dl (PND 21)	1) 23% decrease in NE levels of hypothalamus and striatum; 2) increased turnover of NE in brainstem	Goldman et al. (1980)
	0.2% $Pb(Ac)_2$ in dams' drinking water PND 0-20	-	8% decrease in AChE activity in cerebellum	Gietzen and Woolley (1984)
	0.25% $Pb(Ac)_2$ in dams' drinking water PND 0-35	-	Decline in synthesis and turnover of striatal DA	Govoni et al. (1978a)
	0.25% $Pb(Ac)_2$ in dams' drinking water PND 0-35	-	Increase in DA synthesis in frontal cortex and nuc. accumbens(10-30% and 35-45%, respectively)	Govoni et al. (1979, 1980; Memo et al. (1980a, 1981)
	0.25% $Pb(Ac)_2$ in dams' drinking water PND 0-35	-	1) 50% increase in DA-specific binding to striatal D_2 receptors; 2) 33% decrease in DA-specific binding to nuc. accumbens D_2 receptors	Lucchi et al. (1981)
	0.25% $Pb(Ac_2)$ in dams' drinking water PND 0-56	-	1) Decline in uptake of DA by striated nerve endings 2) Elevated DA uptake in nuc. accumbens	Missale et al. (1984)

TABLE 12-8. (continued)

Subject	Exposure protocol	Peak blood lead level	Observed effect	Reference
	0.25% $Pb(Ac)_2$ dams' drinking water PND 0-56	71 µg/dl (PND 56)	27% decrease in DA-specific binding to pituitary D_2 receptors	Govoni et al. (1984)
	0.25% $Pb(Ac)_2$ in dams' drinking water PND 0-42	87 µg/dl (PND 42)	1) 31% increase in GABA specific binding in cerebellum; 53% increase in GMP activity; 2) 36% decrease in GABA-specific binding in striatum; 47% decrease in GMP activity	Govoni et al. (1978b, 1980)
	0.25% $Pb(Ac)_2$ in dams' drinking water PND 0-21; 0.004% or 0.25% until PND 42	-	1) 12 and 34% elevation of GABA binding in cerebellum for 0.004% and 0.25%, respectively; 2) 20 and 45% decreases in GABA binding in striatum for 0.04 and 0.25%, respectively	Memo et al. (1980b)
	0.5-1% $Pb(Ac)_2$ in drinking water PND 0-60	-	1) Increased sensitivity to seizures induced by GABA blockers; 2) increase in GABA synthesis in cortex and striatum; 3) inhibition of GABA uptake and release by synaptosomes from cerebellum and basal ganglia; 4) 70% increase in GABA-specific binding in cerebellum	Silbergeld et al. (1979, 1980a)
	0.25-1% $Pb(Ac)_2$ in drinking water PND 0-60	72-91 g/dl (PND 21)	1) 40-50% reduction of whole-brain ACh by PND 21; 2) 36% reduction by PND 30 (return to normal values by PND 60)	Modak et al. (1978)
	75 mg $Pb(Ac)_2$/kg b.w./day via gastric intubation PND 2-14	98 µg/dl (PND 15)	1) 20% decline in striatal DA levels at PND 35; 2) 35% decline in striatal DA turnover by PND 35; 3) Transient depression of DA uptake at PND 15; 4) Possible decreased DA terminal density	Jason and Kellogg (1981)

TABLE 12-8. (continued)

Subject	Exposure protocol	Peak blood lead level	Observed effect	Reference
Young rat	2% $Pb(Ac)_2$ in dams' drinking water PND 0-21 then 0.002-0.008% until PND 56	-	1) non-dose-dependent elevations of NE in midbrain (60-90%) and DA and 5-HT in midbrain, striatum and hypothalamus (15-30%); 2) non-dose-dependent depression of NE in hypothalamus and striatum (20-30%).	Dubas et al. (1978)

*Abbreviations:

PND:	postnatal day	DA:	dopamine	ACh:	acetylcholine
$Pb(Ac)_2$:	lead acetate	GABA:	γ-aminobutyric acid	AChE:	acetylcholinesterase
$PbCl_2$:	lead chloride	GMP:	guanosine monophosphate	b.w.:	body weight
NE:	norepinephrine	5-HT:	serotonin		

range from 47 μg/dl (Goldman et al., 1980) to 87 μg/dl (Govoni et al., 1980), a few general observations can be made:

(1) Synthesis, turnover, and uptake of DA and NE are depressed in the striatum, and elevated in midbrain, frontal cortex, and nucleus accumbens. This seems to be paralleled by concomitant increases in DA-specific binding in striatum and decreases in DA-specific binding in nucleus accumbens, possibly involving a specific subset (D_2) of DA receptors (Lucchi et al., 1981). These findings are probably reflective of sensitization phenomena resulting from changes in the availability of neurotransmitter at the synapse.

(2) The findings for pathways utilizing GABA show similar parallels. Increases in GABA synthesis in striatum are coupled with decreases in GABA-specific binding in that region, while the converse holds true for the cerebellum. In these cases, cyclic GMP activity mirrors the apparent changes in receptor function. This increased sensitivity of cerebellar postsynaptic receptors (probably a response to the lead-induced depression of presynaptic function) is likely the basis for the finding that lead-treated animals are more susceptible to seizures induced by GABA-blocking agents such as picrotoxin or strychnine (Silbergeld et al., 1979).

12.4.3.4.2 In vitro studies. Any alterations in the integrity of the blood-brain barrier can have serious consequences for the nervous system, especially in the developing organism. Kolber et al. (1980) examined glucose transport in isolated microvessels prepared from the brains of suckling rats given 25, 100, 200, or 1000 mg lead/kg body weight daily by intragastric gavage. On PND 25, they found that even the lowest dose blocked specific transport sites for sugars and damaged the capillary endothelium. In vitro treatment of the preparation with concentrations of lead as low as 0.1 μM produced the same effects.

Purdy et al. (1981) examined the effects in rats of varying concentrations of lead acetate on the whole-brain synthesis of tetrahydrobiopterin (BH_4), a cofactor for many important enzymes, including those regulating catecholamine (e.g., DA or NE) synthesis. Concentrations of lead as low as 0.01 μM produced a 35 percent inhibition of BH_4 synthesis, while 100 μM inhibited the BH_4 salvage enzyme, dihydropteridine reductase, by 40 percent. This would result in a decreased conversion of phenylalanine to tyrosine and thence to DOPA (the initial steps in dopamine synthesis), as well as decreases in the conversion of trytophan to its 5-hydroxy form (the initial step in serotonin synthesis). These decrements, if occurring in vivo, could not be ameliorated by increased dietary intake of BH_4, as it does not cross the blood-brain barrier.

Lead has also been found to have an inhibitory effect on mitochondrial respiration in the cerebrum and cerebellum of immature or adult rats at concentrations greater than 50 μM (Holtzman et al., 1978). This effect, which was equivalent in both brain regions at both ages studied, is apparently due to an inhibition of nicotinamide adenine dinucleotide (NAD)-linked dehydrogenases within the mitochondrial matrix. These same authors found that this lead-induced effect, which is an energy-dependent process, could be blocked in vitro by addition of ruthenium red to the incubation medium (Holtzman et al., 1980b). In view of the fact that Ca^{2+} uptake and entry into the mitochondrial matrix is also blocked by ruthenium red, it is possible that both lead and Ca^{2+} share the same binding site/carrier in brain mitochondria. These findings are supported by the work of Gmerek et al. (1981) on adult rat cerebral mitochondria, with the exception that they observed respiratory inhibition at 5 μM lead acetate, which is a full order of magnitude lower than the Holtzman et al. (1978, 1980b) studies. Gmerek and co-workers offer the possibility that this discrepancy may have been due to the inadvertent presence of EDTA in the incubation medium used by Holtzman and co-workers.

Organolead compounds have also been demonstrated to have a deleterious effect on cellular metabolism in the nervous system. For example, Grundt and Neskovic (1980) found that concentrations of triethyl lead chloride as low as 5-7 μM caused a 40 percent decrease in the incorporation of SO_4 or serine into myelin galacto-lipids in cerebellar slices from 2-week-old rats. Similarly, Konat and coworkers (Konat and Clausen, 1978, 1980; Konat et al., 1979) observed that 3 μM triethyl lead chloride preferentially inhibited the incorporation of leucine into myelin proteins in brain stem and forebrain slices from 22-day-old rats. This apparent inhibition of myelin protein synthesis was twofold greater than that observed for total protein synthesis (approximately 10 versus 20 percent, respectively). In addition, acute intoxication of these animals by i.p. injection of triethyl lead chloride at 8 mg/kg produced equivalent results accompanied by a 30 percent reduction in total forebrain myelin content.

Interestingly, while a suspension of cells from the forebrain of these animals (Konat et al., 1978) exhibited a 30 percent inhibition of total protein synthesis at 20 μM triethyl lead chloride (the lowest concentration examined), a cell-free system prepared from the same tissue was not affected by triethyl lead chloride concentrations as high as 200 μM. This result, coupled with a similar, although not as severe, inhibitory effect of triethyl lead chloride on oxygen consumption in the cell suspension (20 percent inhibition at 20 μM) would tend to indicate that the inhibition of rat forebrain protein synthesis is related to an inhibition of cellular energy-generating systems.

The effects of organolead compounds on various neurotransmitter systems have been investigated in adult mouse brain homogenates. Bondy et al. (1979a,b) demonstrated that micromolar concentrations (5 μM) of tri-n-butyl lead (TBL) acetate were sufficient not only to cause a 50 percent decline in the high affinity uptake of GABA and DA in such homogenates, but also to stimulate a 25 percent increase in GABA and DA release. These effects were apparently selective for DA neurons at lower concentrations, as only DA uptake or release was affected at 0.1 μM, albeit mildly so. The effect of TBL acetate on DA uptake appears to be specific, as there is a clear dose-response relationship down to 1 μM TBL (Bondy and Agrawal, 1980) for inhibition (0-60 percent) of spiroperidol binding to rat striatal DA receptors. A concomitant inhibition of adenyl cyclase in this dose range (50 percent) suggests that TBL may affect the entire postsynaptic binding site for DA.

12.4.3.5 Accumulation and Retention of Lead in the Brain. All too infrequently, experimental studies of the neurotoxic effects of lead exposure do not report the blood-lead levels achieved by the exposure protocols used. Even less frequently reported are the concomitant tissue levels found in brain or other tissues. From the recent information that is available, however, it is possible to draw some limited conclusions about the relationship of exposure concentrations to blood and brain lead concentrations. Table 12-9 calculates the blood lead/ brain lead ratios found in recent studies where such information was available. It can be seen that, at exposure concentrations greater than 0.2 percent and for exposure periods longer than birth until weaning (21 days in rats), the ratio generally falls below unity. This suggests, that, even as blood lead levels reach a steady state and then fall due to excretion or some other mechanism, lead continues to accumulate in brain.

TABLE 12-9. INDEX OF BLOOD LEAD AND BRAIN LEAD LEVELS FOLLOWING EXPOSURE[a]

Species (strain)	Exposure	Time of assay	Blood lead, µg/dl	Brain lead, µg/100g	Blood:brain lead ratio	Reference
Suckling rat (Charles River-CD)	0.0005% $PbCl_2$ in water PND 0-21	PND 21	12	8	1.5	Bull et al. (1979)
	0.003% $PbCl_2$ in water PND 0-21	PND 21	21	11	1.9	
Suckling rat (Charles River)	0.005% $Pb(Ac)_2$ in water from conception	PND 11	22	3	7.0	Grant et al. (1980)
		PND 30	18	11	1.6	
	0.01% $Pb(Ac)_2$ in water from conception	PND 11	35	7	5.0	
		PND 30	48	22	2.2	
Suckling rat (Charles River-CD)	0.02% $PbCl_2$ in water PND 0-21	PND 21	36	25	1.4	Bull et al. (1979)
Suckling rat (Long-Evans)	0.02% $Pb(Ac)_2$ in water PND 0-21	PND 10	21.7	6.3	3.4	Fox et al. (1979)
		PND 21	25.2	13	1.9	
Suckling rat (Long-Evans)	0.02% $Pb(Ac)_2$ in water from PND 0-21	PND 21	29	29	1.0	Hastings et al. (1979)
Suckling rat (Holtzman-albino)	0.05% $Pb(Ac)_2$ in water PND 0-21	PND 21	12	20	0.6	Goldman et al. (1980)
	0.1% $Pb(Ac)_2$ in water PND 0-21	PND 21	20	50	0.4	
Suckling rat	0.2% $Pb(Ac)_2$ in water PND 0-21	PND 21	65	65	1.0	Hastings et al. (1979)
Suckling rat (Holtzman-albino)	0.2% $Pb(Ac)_2$ in water PND 0-21	PND 21	47	80	0.6	Goldman et al. (1980)
Suckling rat (Long-Evans)	0.2% $Pb(Ac)_2$ in water PND 0-21	PND 10	49.6	19	2.6	Fox et al. (1979)
		PND 21	89.4	82	1.1	

TABLE 12-9. (continued)

Species (strain)	Exposure	Time of assay	Blood lead, µg/dl	Brain lead, µg/100g	Blood:brain lead ratio	Reference
Suckling rat (Long-Evans)	0.2% $Pb(Ac)_2$ in water PND 0-21	PND 21	65.0	53	1.2	Fox et al. (1977)
Suckling rat (Long-Evans)	0.2% $Pb(Ac)_2$ in water PND 0-21	PND 21	65.1	53	1.2	Cooper et al. (1980)
Suckling mice (ICR Swiss albino)	0.25% $Pb(Ac)_2$ in water PND 0-21	PND 21	72	230	0.3	Modak et al. (1978)
Suckling rat (Wistar)	0.2% $Pb(Ac)_2$ in water PND 2-60	PND 30	115^b	84	1.4	Shigeta et al. (1979)
		PND 60	35^b	99	0.4	
	0.5% $Pb(AC)_2$ in water PND 2-60	PND 30	308^b	172	1.8	
		PND 60	73^b	222	0.3	
Suckling rat (Sprague-Dawley)	0.25% $Pb(Ac)_2$ in water from gestation until PND 42	PND 42	87	85	1.0	Govoni et al. (1980)
	0.5% $Pb(Ac)_2$ in water PND 0-21	PND 21	70	280	0.25	
	1% $Pb(Ac)_2$ in water PND 0-21	PND 21	91	270	0.3	
Suckling rat (Wistar albino)	0.5% $Pb(Ac)_2$ in diet PND 0-365	PND 7	70	36	1.9	Mykkänen et al. (1979)
		PND 21	335	127	2.6	
		PND 35	291	124	2.3	
		PND 49	94	122	0.8	
		PND 90	76	123	0.6	Mykkänen et al. (1982)
		PND 180	78	111	0.7	
		PND 365	103	161	0.6	
Suckling rat (Sprague-Dawley)	4% $PbCO_3$ in water PND 0-27	PND 27	---	1.36	---	Wince et al. (1980)

TABLE 12-9. (continued)

Species (strain)	Exposure	Time of assay	Blood lead, µg/dl	Brain lead, µg/100g	Blood:brain lead ratio	Reference
Suckling rat (Sprague-Dawley)	0.1 mg/kg $Pb(Ac)_2$ by gavage PND 3-56	PND 28 PND 42 PND 56	9.5 13.8 12.7	12.1 11.1 10.2	0.78 1.2 1.3	Collins et al. (1984)
Suckling rat (Long-Evans)	25 mg/kg $Pb(Ac)_2$ by gavage PND 2-14	PND 15	50	40	1.3	Jason and Kellogg (1981)
	75 mg/kg $Pb(Ac)_2$ by gavage PND 2-14	PND 15	98	60	1.6	
Young mice (ICR Swiss albino)	0.25% $Pb(Ac)_2$ in water PND 0-60	PND 60	91	410	0.2	Modak et al. (1978)
	0.5% $Pb(Ac)_2$ in water PND 0-60	PND 60	194	360	0.5	
	1% $Pb(Ac)_2$ in water PND 0-60	PND 60	223	810	0.3	
Weanling rats (Long-Evans)	0.0025% $Pb(Ac)_2$ in water from PND 22		18	7	2.6	Cory-Slechta et al. (1985)
	0.005% $Pb(Ac)_2$ in water from PND 22		20	30	0.7	
	0.01% $Pb(Ac)_2$ in water from PND 22		40	50	0.8	
	0.05% $Pb(Ac)_2$ in water from PND 22		100	120	0.8	
Adult rat (Charles River-CD)	0.0005% $Pb(Ac)_2$ in water for 21 days		9	10	0.9	Bull et al. (1979)
	0.003% $Pb(Ac)_2$ in water for 21 days		11	12	0.9	
	0.02% $Pb(Ac)_2$ in water for 21 days		29	100	0.3	

TABLE 12-9. (continued)

Species (strain)	Exposure	Time of assay	Blood lead, µg/dl	Brain lead, µg/100g	Blood:brain lead ratio	Reference
Adult rat (Wistar)	0.15% $Pb(Ac)_2$ in water for 3 months		31	12-18[c]	2.6-1.7[c]	Ewers and Erbe (1980)
	0.4% $Pb(Ac)_2$ in water for 3 months		69	16-34[c]	4.3-2.0[c]	
	1% $Pb(Ac)_2$ in water for 3 months		122	37-72[c]	3.3-1.7[c]	

[a] Abbreviations:
PND: post-natal day
$Pb(Ac)_2$: lead acetate
$PbCl_2$: lead chloride

[b] Expressed as µg Pb/100g blood.

[c] Depending on region.

Further evidence bearing on this was derived from a set of studies by Goldstein et al. (1974), who reported that administration of a wide range of doses of radioactive lead nitrate to one-month-old rats resulted in parallel linear increases in both blood and brain lead levels during the ensuing 24 hours. This suggests that deposition of lead in brain occurs without threshold and that, at least initially, it is proportional to blood lead concentration. However, further studies by Goldstein et al. (1974) followed changes in blood and brain lead concentrations after cessation of lead exposure and found that, whereas blood lead levels decreased dramatically (by an order of magnitude or more) during a 7-day period, brain lead levels remained essentially constant over the one-week postexposure period. Thus, with even intermittent exposures to lead, it is not unexpected that brain concentrations would tend to remain the same or even to increase although blood lead levels may have returned to "normal" levels. Evidence confirming this comes from findings of two studies: (1) Hammond (1971), showing that EDTA administration causing marked lead excretion in urine of young rats did not significantly lower brain lead levels in the same animals; and (2) Goldstein et al. (1974), showing that although EDTA prevented the in vitro accumulation of lead into brain mitochondria, if lead was added first EDTA was ineffective in removing lead from the mitochondria. These results, overall, indicate that, although lead may enter the brain in rough proportion to circulating blood lead concentrations, it is then taken up by brain cells and tightly bound into certain subcellular components (such as mitochondrial membranes) and retained there for quite long after initial external exposure ceases and blood lead levels markedly decrease. This may help to account for the persistence of neurotoxic effects of various types noted above long after the cessation of external lead exposure.

The uptake of lead into specific neural and non-neuronal elements of the brain has also been studied and provides insight into possible morphological correlates of certain lead effects discussed above and below as being observed *in vivo* or *in vitro*. For example, Collins et al. (1984) observed preferential accumulation of lead in the hippocampus of suckling rats fed 0.1 mg/kg $Pb(Ac)_2$ per day by gastric intubation from PND 3-56. In another study, Stumpf et al. (1980), via autoradiographic localization of ^{210}Pb, found that ependymal cells, glial cells, and endothelial cells of brain capillaries concentrate and retain lead above background levels for several days after injections of tracer amounts of the elements. These cells are non-neural elements of brain important in the maintenance of "blood-brain barrier" functions, and their uptake and retention of lead, even with tracer doses, provides evidence of a morphological basis by which lead effects on blood-brain barrier functions may be exerted. Again, the retention of lead in these non-neuronal elements for at least several days after original exposure points towards the plausibility of lead exerting effects on blood-brain barrier functions long after external exposure ceases and blood lead levels decrease back toward normal levels. Uptake and concentration of lead in the nuclei of some cortical neurons even several days after administration of only a tracer dose of ^{210}Pb was also observed by Stumpf et al. (1980) and provide yet another plausible morphological basis by which neurotoxic effects might be exerted by lead long after external exposure terminates and blood lead levels return to apparently "normal" levels.

12.4.4 Integrative Summary of Human and Animal Studies of Neurotoxicity

An assessment of the impact of lead on human and animal neurobehavioral function raises a number of issues. Among the key points addressed here are the following: (1) the internal exposure levels, as indexed by blood lead levels, at which various adverse neurobehavioral effects occur; (2) the reversibility of such deleterious effects; and (3) the populations that appear to be most susceptible to neural damage. In addition, the question arises as to the utility of using animal studies to draw parallels to the human condition.

12.4.4.1 Internal Exposure Levels at Which Adverse Neurobehavioral Effects Occur. Markedly elevated blood lead levels are associated with neurotoxic effects (including severe, irreversible brain damage as indexed by the occurrence of acute and/or chronic encephalopathic symptoms) in both humans and animals. For most adult humans, such damage typically does not occur until blood lead levels exceed 120 µg/dl. Evidence does exist, however, for acute encephalopathy and death occurring in some human adults at blood lead levels below 120 µg/dl, down to about 100 μg/dl. In children, effective blood lead levels for producing encephalopathy or death are somewhat lower, encephalopathy signs and symptoms having been reported for some children at blood lead levels as low as 80-100 μg/dl.

It should be emphasized that, once encephalopathy occurs, death is not an improbable outcome, regardless of the quality of medical treatment available at the time of acute crisis. In fact, certain diagnostic or treatment procedures themselves tend to exacerbate matters and push the outcome toward fatality if the nature and severity of the problem are not fully recognized or properly diagnosed. It is also crucial to note the rapidity with which acute encephalopathic symptoms can develop or death can occur in apparently asymptomatic individuals or in those apparently only mildly affected by elevated body burdens of lead. It is not unusual for rapid deterioration to occur, with convulsions or coma suddenly appearing and with progression to death within 48 hours. This strongly suggests that, even in apparently asymptomatic individuals, rather severe neural damage probably exists at high blood lead levels although such damage is not yet overtly manifested in obvious encephalopathic symptoms. This conclusion is further supported by numerous studies showing that children with high blood lead levels (over 80-100 μg/dl), but not observed to manifest acute encephalopathic symptoms, are permanently cognitively impaired, as are most children who survive acute episodes of frank lead encephalopathy.

Growing evidence indicates that subencephalopathic lead intoxication in adults causes various overt neurological signs and symptoms at blood lead levels as low (40-60 μg/dl) as those at which other overt manifestations (e.g., gastrointestinal symptoms) of lead intoxication have been detected. In addition, among apparently asymptomatic, non-overtly lead-intoxicated adults, often more subtle (but important) central and peripheral nervous system effects, e.g. slowed nerve conduction velocities, have been observed at blood lead levels as low as 30 μg/dl.

Other evidence confirms that various types of neural dysfunction exist in apparently asymptomatic children across a broad range of blood lead levels. The body of studies on low- or moderate-level lead effects on neurobehavioral functions, as summarized in Table 12-2, presents a rather impressive array of data pointing to that conclusion. At high exposure levels, several studies point to average 5-point IQ decrements in asymptomatic children at average blood levels of 50-70 μg/dl. Other evidence is indicative of average IQ decrements of up to 4 points being associated with blood levels in a 30-50 μg/dl range. Below 30 μg/dl, the evidence for IQ decrements is mixed, with some studies showing no significant associations with lead once other confounding factors are controlled. Still, the 1-2 point differences in IQ generally seen with blood lead levels in the 15-30 μg/dl range are suggestive of small lead effects that are typically dwarfed by other social factors. Moreover, the highly significant linear relationship between IQ and blood lead over the range of 6 to 47 μg/dl found in low-SES Black children indicates that IQ effects may be detected without evident threshold even at these low levels, at least in this population of children. In addition, other behavioral (e.g., reaction time, psychomotor performance) and electrophysiological (altered EEG patterns, evoked potential measures, and peripheral nerve conduction velocities) are consistent with a dose-response function relating neurotoxic effects to lead exposure levels as low as 15-30 μg/dl and possibly lower. Although the comparability of blood lead concentrations across species is uncertain (see discussion below), animal studies show neurobehavioral effects in rats and monkeys at maximal blood lead levels below 20 μg/dl; some studies demonstrate residual effects long after lead exposure has terminated and blood lead levels have returned to approximately normal levels.

Timing, type, and duration of exposure are important factors in both animal and human studies. It is often uncertain whether observed blood lead levels represent the levels that were responsible for observed behavioral deficits. Monitoring of lead exposures in pediatric subjects in all cases has been highly intermittent or non-existent during the period of life preceding neurobehavioral assessment. In most studies of children, only one or two blood lead values are provided per subject. Tooth lead may be an important cumulative exposure index; but its modest, highly variable correlation to blood lead, FEP, or external exposure levels makes findings from various studies difficult to compare quantitatively. The complexity of the many important covariates and their interaction with dependent measures of modest validity, e.g., IQ tests, may also account for many of the discrepancies among the different studies.

The precise medical or health significance of the neuropsychological and electrophysiological effects associated with low-level lead exposure as reported in the above studies is difficult to state with confidence at this time. Observed IQ deficits and other behavioral changes, although statistically significant in some studies, tend to be relatively small as reported by the investigators, but nevertheless may still affect the intellectual development, school performance, and social development of the affected children sufficiently to be regarded as adverse. This would be especially true if such impaired intellectual development or school performance and disrupted social development were reflective of persisting, long-term effects of low-level lead exposure in early childhood. Although the issue of persistence of such lead effects remains to be more clearly resolved, some study results reviewed above suggest that significant low-level lead-induced neurobehavioral and electrophysiological effects may, in fact, persist at least into later childhood. Animal studies also demonstrate long-term neurobehavioral effects of relatively moderate- or low-level lead exposure, even after blood lead concentrations have dropped to nearly normal levels.

12.4.4.2 The Question of Irreversibility. Little research on humans is available on persistence of effects. Some work suggests the possibility of reversing mild forms of peripheral neuropathy in lead workers, but little is known regarding the reversibility of lead effects on central nervous system function in humans. A series of studies on a group of lead-exposed children indicate persistent relationships between blood lead and altered slow wave cortical potentials at two- and five-year follow-ups. However, IQ deficits in the same group of subjects were no longer evident at the five-year follow-up. Some work suggests that other measures of classroom performance may be more sensitive indicators of lead-induced effects in older children. Prospective longitudinal studies on the developmental effects of lead are needed to answer questions on the persistence or reversibility of neurotoxic effects of early lead exposure.

Various animal studies provide evidence that alterations in neurobehavioral function may be long-lived, with such alterations being evident long after blood lead levels have returned to control levels. These persistent effects have been demonstrated in monkeys as well as rats under a variety of learning performance test paradigms. Such results are also consistent with morphological, electrophysiological, and biochemical studies on animals that suggest lasting changes in synaptogenesis, dendritic development, myelin and fiber tract formation, ionic mechanisms of neurotransmission, and energy metabolism.

12.4.4.3 Early Development and Susceptibility to Neural Damage. On the question of early childhood vulnerability, the neurobehavioral data are consistent with morphological and biochemical studies of the susceptibility of the heme biosynthetic pathway to perturbation by lead. Various lines of evidence suggest that the order of susceptibility to neurotoxic effects of lead is: young > adult, and female > male. Animal studies also have pointed to the perinatal period of ontogeny as a particularly critical time for a variety of reasons: (1) it is a period of rapid development of the nervous system; (2) it is a period where good nutrition is particularly critical; and (3) it is a period where the caregiver environment is vital to normal development. However, the precise boundaries of a critical period for lead exposure are not yet clear and may vary depending on the species and function or endpoint that is being assessed. One analysis of lead-exposed children suggests that differing effects on cognitive performance may be a function of the different ages at which children are subjected to neurotoxic exposures. Nevertheless, there is general agreement that human infants and toddlers below the age of three years are at special risk because of *in utero* exposure, increased opportunity for exposure because of normal mouthing behavior of lead-containing objects, and increased rates of lead absorption due to various factors, e.g., iron and calcium deficiencies.

12.4.4.4 Utility of Animal Studies in Drawing Parallels to the Human Condition. Animal models are used to shed light on questions where it would be impractical or ethically unacceptable to use human subjects. This is particularly true in the case of exposure to environmental toxins such as lead. In the case of lead, it has been most effective and convenient to expose developing animals via their mothers' milk or by gastric gavage, at least until weaning. Very often, the exposure is continued in the water or food for some time beyond weaning. This approach does succeed in simulating at least two features commonly found in human exposure: oral intake and exposure during early development. The preweaning postnatal period in rats and mice is of particular relevance in terms of parallels with the first two years or so of human brain development.

Studies using rodents and monkeys have provided a variety of evidence of neurobehavioral alteration induced by lead exposure. In most cases these effects suggest impairment in "learning," i.e., the process of appropriately modifying one's behavior in response to information from the environment. Such behavior involves the ability to receive, process, and remember information in various forms. Some studies indicate behavioral alterations of a more basic type, such as delayed development of certain reflexes. Other evidence suggests changes affecting rather complex behavior in the form of social interactions.

Most of the above effects are evident in rodents and monkeys with blood lead levels exceeding 30 μg/dl, but some effects on learning ability are apparent even at maximum blood lead exposure levels below 20 μg/dl. Can these findings with animals be generalized to humans? Given differences between humans, rats, and monkeys in heme chemistry, metabolism, and other aspects of physiology and anatomy, it is difficult to state what constitutes an equivalent internal exposure level, much less an equivalent external exposure level (see Hammond et al. (1985) for a discussion of this). For example, is a blood lead level of 30 μg/dl in a suckling rat equivalent to 30 μg/dl in a three-year-old child? Until an answer is available for this question, i.e., until the function describing the relationship of exposure indices in different species is available, the utility of animal models for deriving dose-response functions relevant to humans will be limited.

Questions also exist regarding the comparability of neurobehavioral effects in animals with human behavior and cognitive function. One difficulty in comparing behavioral endpoints such as locomotor activity is the lack of a consistent operational definition. In addition to the lack of standardized methodologies, behavior is notoriously difficult to "equate" or compare meaningfully across species because behavioral analogies do not demonstrate behavioral homologies. Thus, it is improper to assume, without knowing more about the responsible underlying neurological structures and processes, that a rat's performance on an operant conditioning schedule or a monkey's performance on a stimulus discrimination task necessarily corresponds directly to a child's performance on a cognitive function test. Nevertheless, interesting parallels in hyper-reactivity and increased response variability do exist between different species, and deficits in performance by mammalian animals on various tasks are probably indicative of altered CNS functions, which are likely to parallel some type of altered CNS function in humans as well.

In terms of morphological findings, there are reports of hippocampal lesions in both lead-exposed rats and humans that are consistent with a number of independent behavioral findings suggesting an impaired ability to respond appropriately to altered contingencies for rewards. That is, subjects with hippocampal damage tend to persist in certain patterns of behavior even when changed conditions make the behavior inappropriate; the same sort of tendency seems to be common to a number of lead-induced behavioral effects, including deficits in passive avoidance, operant extinction, visual discrimination, and various other discrimination reversal tasks. Other morphological findings in animals, such as demyelination and glial cell decline, are comparable to human neuropathologic observations only at relatively high exposure levels.

Another neurobehavioral endpoint of interest in comparing human and animal neurotoxicity of lead is electrophysiological function. Alterations of electroencephalographic patterns and cortical slow wave voltage have been reported for lead-exposed children, and various electrophysiological alterations both *in vivo* (e.g., in rat visual evoked response) and *in vitro* (e.g., in frog miniature endplate potentials) have also been noted in laboratory animals. Thus, far, however, these lines of work have not converged sufficiently to allow for much in the way of definitive conclusions regarding electrophysiological aspects of lead neurotoxicity.

Biochemical approaches to the experimental study of lead effects on the nervous system have been basically limited to laboratory animal subjects. Although their linkage to human neurobehavioral function is at this point somewhat speculative, such studies do provide insight on possible neurochemical intermediaries of lead neurotoxicity. No single neurotransmitter system has been shown to be particularly sensitive to the effects of lead exposure; lead-induced alterations have been demonstrated in various neurotransmitters, including dopamine, norepinephrine, serotonin, and gamma-aminobutyric acid. In addition, lead has been shown to have subcellular effects in the central nervous system at the level of mitochondrial function and protein synthesis. In particular, some work has indicated that delays seen in cortical synaptogenesis and metabolic maturation following prenatal lead exposure may well underlie the delayed development of exploratory and locomotor function seen in other studies of the neurobehavioral effects of lead. Further studies on the correlation between human blood lead values and lead-induced disruptions of tetrahydrobiopterin metabolism indicate that subsequent interference with neurotransmitter formation may be linked to small reductions in IQ scores.

Given the difficulties in formulating a comparative basis for internal exposure levels among different species, the primary value of many animal studies, particularly *in vitro* studies, may be in the information they can provide on basic mechanisms involved in lead neurotoxicity. A number of key *in vitro* studies are summarized in Table 12-10. These studies show that significant, potentially deleterious effects on nervous system function occur at *in situ* lead concentrations of 5 μM and possibly lower. This suggests that, at least intracellularly or on a molecular level, there may exist essentially no threshold for certain neurochemical effects of lead. The relationship between blood lead levels and lead concentrations at extra- or intracellular sites of action, however, remains to be determined.

Despite the problems in generalizing from animals to humans, both the animal and the human studies show considerable internal consistency in that they both support a continuous dose-response functional relationship between lead and neurotoxic biochemical, morphological, electrophysiological, and behavioral effects.

TABLE 12-10. SUMMARY OF KEY STUDIES OF IN VITRO LEAD EXPOSURE*

Preparation	Exposure concentration	Results	Reference
Adult rat brain	0.1 μM $Pb(Ac)_2$	35% inhibition of whole-brain BH_4 synthesis	Purdy et al. (1981)
Isolated microvessels from rat brain	0.1 μM $Pb(Ac)_2$	Blockade of sugar-specific transport sites in capillary endothelial cells	Kolber et al. (1980)
Adult mouse brain homogenate	0.1-5 μM TBL	1) 50% decline in high affinity uptake of DA; 2) 25% increase in release of DA	Bondy et al. (1979a,b)
Adult rat striatum	1-5 μM TBL	0-60% inhibition of spiroperidal binding to DA receptors	Bondy and Agrawal (1980)
Embryonic chick brain cell culture	3 μM $(Et_3Pb)Cl_2$	50% reduction in no. of cells exhibiting processes	Grundt et al. (1981)
Brainstem and forebrain slices from PND-22 rats	3 μM $(Et_3Pb)Cl_2$	Inhibition of leucine incorporation into myelin proteins	Konat and Clausen (1978, 1980); Konat et al. (1979)
Adult rat cerebellar homogenates	3-5 μM Pb^{2+}	Inhibition of adenylate cyclase activity	Taylor et al. (1978)
Adult rat cerebellar mitochondria	5 μM $Pb(Ac)_2$	Inhibition of respiration	Gmerek et al. (1981)
Adult frog nerve/muscle preparation	5 μM Pb^{2+}	Increase in frequency of MEPP's (indicative of depression of synaptic transmission)	Kolton and Yaari (1982)
Isolated, perfused bullfrog retina	5 μM Pb^{2+}	Depression of rod (but not cone) receptor potentials	Fox and Sillman (1979)

TABLE 12-10. (continued)

Preparation	Exposure concentration	Results	Reference
Cerebellar slices from PND-14 rats	5-7 μM $(Et_3Pb)Cl_2$	Inhibition of incorporation of SO_4 and serine into myelin galactolipids	Grundt and Neskovic (1980)
In oculo culture of cerebellar tissue from PND-15 rats	5-10 μM Pb^{2+}	"Disinhibition" of NE-induced inhibition of spontaneous activity in Purkinje cells	Taylor et al. (1978)
Cell suspension from forebrain of PND-22 rats	20 μM $(Et_3Pb)Cl_2$	30% inhibition of total protein synthesis	Konat et al. (1978)
Adult rat cerebral and cerebellar mitochondria	50 μM $Pb(Ac)_2$	Inhibition of respiration	Holtzman et al. (1978, 1980b)
Adult rat caudate synaptosomes	50 μM $PbCl_2$	8-fold increase in binding of Ca^{2+} to mitochondria (effectively increases Ca^{2+} gradient across terminal membrane, thus increasing synaptic transmission without altering firing rates)	Silbergeld and Adler (1978)
Capillary endothelial cells from rat cere-cortex	100 μM $Pb(Ac)_2$	Pb preferentially sequestered in mitochondria like Ca^{2+} (possible basis for Pb-induced disruption of transmembrane Ca^{2+} transport)	Silbergeld et al. (1980b)

*Abbreviations:

PND: postnatal day
$Pb(Ac)_2$: lead acetate
$PbCl_2$: lead chloride
Et_3Pb: triethyl lead
TBL: tri-n-butyl lead
DA: dopamine
NE: norepinephrine
BH_4: tetrahydrobiopterin
MEPP's: miniature endplate potentials

12.5 EFFECTS OF LEAD ON THE KIDNEY

12.5.1 Historical Aspects

The first description of renal disease due to lead was published by Lancereaux (1862). In a painter with lead encephalopathy and gout, Lancereaux noted tubulo-interstitial disease of the kidneys at autopsy. Distinctions between glomerular and tubulo-interstitial forms of kidney disease were not, however, clearly defined in the mid-nineteenth century. Ollivier (1863) reported observations in 37 cases of lead poisoning with renal disease and thus introduced the idea that lead nephropathy was a proteinuric disease, a confusion with primary glomerular disease that persisted for over a century. Under the leadership of Jean Martin Charcot, interstitial nephritis characterized by meager proteinuria in lead poisoning was widely publicized (Charcot, 1874; Charcot and Gombault, 1881) but not always appreciated by contemporary physicians (Danjoy, 1864; Geppert, 1882; Lorimer, 1886).

More than ninety years ago, the English toxicologist Oliver (1885, 1891) distinguished acute effects of lead on the kidney from lead-induced chronic nephropathy. Acute renal effects of lead were seen in persons dying of lead poisoning and were usually restricted to non-specific changes in the renal proximal tubular lining cells. Oliver noted that a "true interstitial nephritis" developed later, often with glomerular involvement.

In an extensive review of the earlier literature, Pejić (1928) emphasized that changes in the proximal tubules, rather than the vascular changes often referred to in earlier studies (Gull and Sutton, 1872), constitute the primary injury to the kidney in lead poisoning. Many subsequent studies have shown pathological alterations in the renal tubule with onset during the early or acute phase of lead intoxication. These include the formation of inclusion bodies in nuclei of proximal tubular cells (Blackman, 1936) and the development of functional defects as well as ultrastructural changes, particularly in renal tubular mitochondria. Wedeen (1984) has extensively reviewed the history of lead poisoning and its relationship to kidney disease.

12.5.2 Lead Nephropathy in Childhood

Dysfunction of the proximal tubule was first noted as glycosuria in the absence of hyperglycemia in childhood pica (McKhann and Vogt, 1926). Later it was shown that the proximal tubule transport defect included aminoaciduria (Wilson et al., 1953). Subsequently, Chisolm et al. (1955) found that the full Fanconi syndrome was present: glycosuria, aminoaciduria, phosphaturia (with hypophosphatemia), and rickets. Proximal tubular transport defects appeared only when blood lead levels exceeded 80 µg/dl. Generalized aminoaciduria was seen more consistently in Chisolm's (1962, 1968) studies than were other manifestations of renal dysfunction. The condition was related to the severity of clinical toxicity, with the complete Fanconi syndrome occurring in encephalopathic children when blood lead concentrations exceeded

150 μg/dl (National Academy of Sciences, 1972). Children who were under three years of age excreted 4-8.9 mg of lead chelate during the first day of therapy with $CaNa_2EDTA$ at 75 mg/kg per day. The aminoaciduria disappeared after treatment with chelating agents and clinical remission of other symptoms of lead toxicity (Chisolm, 1962). This is an important observation relative to the long-term or chronic effects of lead on the kidney.

In a group of children with slight lead-related neurological signs reported by Pueschel et al. (1972), generalized aminoaciduria was found in 8 of 43 children with blood lead levels of 40-120 μg/dl. Blood lead values in the children with aminoaciduria were not specifically provided but presumably were among the highest found. It should be noted that the children reported to have aminoaciduria in this study were selected because of a blood lead level of ≧50 μg/dl or a provocative chelation test of >500 μg of lead chelate per 24 hours.

Although children are considered generally to be more susceptible than adults to the toxic effects of lead, the relatively sparse literature on childhood lead nephropathy probably reflects a greater clinical concern with the life-threatening neurologic symptoms of lead intoxication than with the transient Fanconi syndrome.

12.5.3 Lead Nephropathy in Adults

There are various lines of evidence in the literature that prolonged lead exposure in humans can result in chronic lead nephropathy in adults. This evidence is reviewed below in terms of six major categories: (1) lead nephropathy following childhood lead poisoning; (2) "moonshine" lead nephropathy; (3) occupational lead nephropathy; (4) lead and gouty nephropathy; (5) lead and hypertensive nephrosclerosis; and (6) general population studies.

Although a variety of methods have been used to assess body burdens of lead, the EDTA lead-mobilization test has emerged as the most reliable index of cumulative lead stores (see Chapter 10, Section 10.3.3). The reliability of this test is apparent under various conditions. For example, Leckie and Tompsett (1958) showed that increasing the dosage of $CaNa_2EDTA$ above 2 g/day intravenously had little effect on the amount of lead chelate excreted by adults. They observed little difference in chelatable lead excretion when 1 g was compared with 2 g intravenously. Similarly, the magnitude of lead chelated when 1 g is given intravenously or 2 g intramuscularly (over 12 hr) appears to be the same (Albahary et al., 1961; Emmerson, 1963; Wedeen et al., 1975). Adult control subjects without undue lead absorption excrete less than 650 μg lead chelate during the first post-injection day if renal function is normal, or over four days if renal function is severely reduced. Thus, in adults, urinary excretion of lead chelate in excess of about 600 μg over one or more days following 1-3 g $CaNa_2EDTA$ administered intravenously or intramuscularly is considered indicative of excessive past lead absorption. The level of reduction of glomerular filtration rate at which the EDTA lead-mobilization test is no longer reliable has not been precisely defined but probably exceeds a reduction of 85 percent (serum creatinine concentrations in excess of about 6 mg/dl).

12.5.3.1 Lead Nephropathy Following Childhood Lead Poisoning. Reports from Queensland, Australia (Gibson et al., 1893; Nye, 1933; Henderson, 1954; Emmerson, 1963) point to a strong association between severe childhood lead poisoning (including central nervous system symptoms) and chronic nephritis in early adulthood. The Australian children sustained acute lead poisoning because the houses around Brisbane were painted with white lead, which the children ingested by direct contamination of their fingers or by drinking lead-sweetened rain water as it flowed over the weathered surfaces. Two fingers brushed against the powdery paint were shown to pick up about 2 mg of lead (Murray, 1939). Henderson (1954) followed up 401 untreated children who had been diagnosed as having lead poisoning in Brisbane between 1915 and 1935. Of these 401 subjects, death certificates revealed that 165 had died under the age of 40, 108 from nephritis or hypertension. This is greatly in excess of expected probabilities. Information was obtained from 101 of the 187 survivors, and 17 of these had hypertension and/or albuminuria.

The Australian investigators also established the validity of the EDTA lead-mobilization test for the detection of excessive past lead absorption and further demonstrated that the body lead stores were retained primarily in bone (Emmerson, 1963; Henderson, 1954; Inglis et al., 1978). Bone lead concentrations averaged 94 µg/g wet weight in the young adults dying of lead nephropathy in Australia (Henderson and Inglis, 1957; Inglis et al., 1978), compared with mean values ranging from 14 to 23 µg/g wet weight in bones from non-exposed individuals (Barry, 1975; Emmerson, 1963; Gross et al., 1975; Wedeen, 1982).

Attempts to confirm the relationship between childhood lead intoxication and chronic nephropathy have not been successful in at least two studies in the United States. Most children in the United States who suffer from overt lead toxicity do so early in childhood, between the ages of 1 and 4, the source often being oral ingestion of flecks of wall paint and plaster containing lead. Tepper (1963) found no evidence of increased chronic renal disease in 139 persons with a well-documented history of childhood plumbism 20-35 years earlier at the Boston Children's Hospital. The total study population comprised 165 patients (after review of 524 case records) who met any two of the following criteria: 1) a definite history of pica or use of lead nipple shields; 2) X-ray evidence of lead-induced skeletal alterations; or 3) characteristic symptoms of lead toxicity. No uniform objective measure of lead absorption was reported. In 42 of the 139 subjects in question, clinical tests of renal function were performed and included urinalysis, endogenous creatinine clearance, urine culture, urine concentrating ability, 24-hour protein excretion, and phenolsulfonphthalein excretion. Only one patient was believed to have died of lead nephropathy; three with creatinine clearances under 90 ml/min were said to have had inadequate urine collections. Insufficient details concerning past lead absorption and patient selection were provided to permit generalized conclusions from this report.

Chisolm et al. (1976) also found no evidence of renal disease in 55 adolescents known to have been treated for lead intoxication 11-16 years earlier. This U.S. study was carried out on adolescents between 12 and 22 years of age in the late 1960s. During acute toxicity in early childhood, blood lead levels had ranged from 100 to 650 µg/dl; all had received immediate chelation therapy. Follow-up chelation tests performed with 1 g EDTA i.m. (with procaine) approximately a decade later resulted in 24-hour lead-chelate excretion of less than 600 µg in 45 of 52 adolescents. Thus, an important distinction between the Australian group and those patients in the United States studied by Chisolm et al. (1976) is that none of the latter subjects showed evidence of increased residual body lead burden by the EDTA lead-mobilization test. The absence of renal disease (as judged by routine urinalysis, blood serum urea nitrogen, serum uric acid, and creatinine clearance) led Chisolm et al. to suggest that lead toxicity in the Australian children may have been of a different type, with a more protracted course than that experienced by the American children. On the other hand, chelation therapy of the American children may have removed lead stored in bone and thus prevented the development of renal failure later in life.

12.5.3.2 "Moonshine" Lead Nephropathy. In the United States, chronic lead nephropathy in adults was first noted among illicit whiskey consumers in the southeastern states. The prerevolutionary tradition of homemade whiskey ("moonshine") was modernized during the Prohibition era for large-scale production. The copper condensers traditionally used in the illegal stills were replaced by truck radiators with lead-soldered parts. Illegally produced whiskey might contain up to 74 mg of lead per liter (Eskew et al., 1961). The enormous variability in moonshine lead content has recently been reiterated in a study of 12 samples from Georgia, of which five contained less than 10 µg/l but one contained 5.3 mg/l (Gerhardt et al., 1980).

Renal disease often accompanied by hypertension and gout was common among moonshiners (Eskew et al., 1961; Morgan et al., 1966; Ball and Sorensen, 1969). These patients usually sought medical care because of symptomatic lead poisoning characterized by colic, neurological disturbances, and anemia, although more subtle cases were sometimes detected by use of the i.v. EDTA lead-mobilization test (Morgan, 1968; Morgan and Burch, 1972). While acute symptomatology, including azotemia, sometimes improved during chelation therapy, residual chronic renal failure, gout, and hypertension frequently proved refractory, thus indicating underlying chronic renal disease superimposed on acute renal failure due to lead (Morgan, 1975).

12.5.3.3 Occupational Lead Nephropathy. Although rarely recognized in the United States (Brieger and Rieders, 1959; Anonymous, 1966; Greenfield and Gray, 1950; Johnstone, 1964; Kazantzis, 1970; Lane, 1949; Malcolm, 1971; Mayers, 1947), occupational lead nephropathy, often in association with gout and hypertension, was widely identified in Europe as a sequela to overt lead intoxication in the industrial setting (Albahary et al., 1961, 1965; Cramer et al., 1974; Danilović, 1958; Galle and Morel-Maroger, 1965; Lejeune et al., 1969; Lilis et al., 1967, 1968; Radosević et al., 1961; Radulescu et al., 1957; Richet et al., 1964, 1966; Tara and Francon, 1975; Vigdortchik, 1935). Some of the more important recent studies are summarized here.

Richet et al. (1964) reported renal findings in eight lead workers, all of whom had repeated episodes of lead poisoning, including colic. Intravenous EDTA lead-mobilization tests ranged from 587 to 5930 µg lead-chelate excretion per 24 hours. Four of these men had reduced glomerular filtration rates, one had hypertension with gout, one had hypertension alone, and one had gout alone. Proteinuria exceeded 200 mg/day in only one patient. Electron microscopy showed intranuclear and cytoplasmic inclusions and ballooning of mitochondria in proximal tubule cells. The presence of intranuclear inclusion bodies is helpful in establishing a relationship between renal lesions and lead toxicity, but inclusion bodies are not always present in persons with chronic lead nephropathy (Cramer et al., 1974; Wedeen et al., 1975, 1979).

Richet et al. (1966) subsequently recorded renal findings in 23 symptomatic lead workers in whom blood lead levels ranged from 30 to 87 µg/dl. Six had diastolic pressures over 90 mm Hg, three had proteinuria exceeding 200 mg/day, and five had gout. In 5 of 21 renal biopsies, glomeruli showed minor hyalinization, but two cases showed major glomerular disease. Interstitial fibrosis and arteriolar sclerosis were seen in all but two biopsies. Intranuclear inclusion bodies were noted in 13 cases. Electron microscopy showed loss of brush borders, iron-staining intracellular vacuoles, and ballooning of mitochondria in proximal tubule epithelial cells.

Effective renal plasma flow, as measured by plasma clearance of para-aminohippurate (C_{pah}), was determined in 14 lead-poisoned Rumanian workers before and after chelation therapy by Lilis et al. (1967). C_{pah} increased from a pre-treatment mean of 428 ml/min (significantly less than the control mean of 580 ml/min) to a mean of 485 ml/min after chelation therapy ($p < 0.02$). However, no significant increase in glomerular filtration rate (as determined by endogenous creatinine clearance) was found. Lilis et al. (1967) interpreted the change in effective renal plasma flow as indicating reversal of the renal vasoconstriction that accompanied acute lead toxicity. Although neither blood lead concentrations nor long-term follow-up observations of renal function were reported, it seems likely that most of these patients suffered from acute, rather than chronic, lead nephropathy.

In a subsequent set of 102 cases of occupational lead poisoning studied by Lilis et al. (1968), seven cases of clinically verified chronic nephropathy were found. In this group, endogenous creatinine clearance was less than 80 ml/min two weeks or more after the last episode of lead colic. The mean blood lead level approximated 80 µg/dl (range: 42-141 µg/dl.) All patients excreted more than 10 mg lead chelate over 5 days during therapy consisting of 2 g $CaNa_2EDTA$ i.v. daily. Nephropathy was more common among those exposed to lead for more than 10 years than among those exposed for less than 10 years. Most of the Rumanian lead workers had experienced lead colic, and 13 of 17 had persistent hypertension that followed the appearance of renal failure by several years. Proteinuria was absent except in two individuals who excreted 250 and 500 mg/l. Hyperuricemia was not evident in the absence of azotemia. In both studies by Lilis et al. (1967, 1968), reduced urea clearance preceded reduced creatinine clearance.

Cramer et al. (1974) examined renal biopsies from five lead workers exposed for 0.5-20 years in Sweden. Their blood lead levels ranged from 71 to 138 µg/dl, with glomerular filtration rates ranging from 65 to 128 ml/min, but C_{pah} exceeding 600 ml/min in all. Although plasma concentrations of valine, tyrosine, and phenylalanine were reduced, excretion of these amino acids was not significantly different from controls. A proximal tubular reabsorptive defect might, therefore, have been present without increased amino acid excretion because of low circulating levels: increased fractional excretion may have occurred without increased absolute amino acid excretion. Albuminuria and glycosuria were not present. Glomeruli were normal by electron microscopy. Intranuclear inclusions in proximal tubules were found in two patients with normal GFRs, and peritubular fibrosis was present in the remaining three patients who had had the longest occupational exposure (4-20 years).

Wedeen et al. (1975, 1979) reported on renal dysfunction in 140 occupationally exposed men. These investigators used the EDTA lead-mobilization test (1 g $CaNa_2EDTA$ with 1 ml of 2 percent procaine given i.m. twice, 8-12 hr apart) to detect workers with excessive body lead stores. In contrast to workers with concurrent lead exposure (Alessio et al., 1979), blood lead measures have proven unsatisfactory for detection of past lead exposure (Baker et al., 1979; Havelda et al., 1980; Vitale et al., 1975). Of the 140 workers tested, 113 excreted 1000 µg or more of lead-chelate in 24 hr compared with a normal upper limit of 650 µg/day (Albahary et al., 1961; Emmerson, 1973; Wedeen et al., 1975). Glomerular filtration rates (GFR) measured by ^{125}I-iothalamate clearance in 57 men with increased mobilizable lead revealed reduced renal function in 21 (GFR less than 90 ml/min per 1.73 m^2 body surface area). When workers over age 55 or with gout, hypertension, or other possible causes of renal disease were excluded, 15 remained who had previously unsuspected lead nephropathy. Their GFRs ranged between 52 and 88 ml/min per 1.73 m^2. Only three of the men with occult renal failure had ever experienced symptoms attributable to lead poisoning. Of the 15 lead nephropathy patients, one had a blood lead level over 80 µg/dl, three repeatedly had blood levels under 40 µg/dl, and eleven had blood levels between 40 and 80 µg/dl at the time of the study. Thus, blood lead levels were poorly correlated with degree of renal dysfunction. The failure of blood lead level to predict the presence of lead nephropathy probably stems from the independence of blood lead from cumulative bone lead stores (Gross, 1981; Saenger et al., 1982a,b).

Percutaneous renal biopsies from 12 of the lead workers with reduced GFRs revealed focal interstitial nephritis in six. Non-specific changes were present in proximal tubules, including loss of brush borders, deformed mitochondria, and increased lysosomal bodies. Intranuclear inclusion bodies were not found in the renal biopsies from these men who had experienced long-term occupational exposure and who had had chelation tests shortly before biopsy. In experimental animals, chelation results in the rapid disapperance of lead-induced intranuclear inclusions (Goyer and Wilson, 1975). The detection of a variety of immunoglobulin deposits by fluorescent microscopy suggests (but does not prove) the possibility that some stages of lead nephropathy in adults may be mediated by immune mechanisms.

Eight patients with pre-azotemic occupational lead nephropathy were treated with 1 g $CaNa_2EDTA$ (with procaine) i.m. three times weekly for 6-50 months. In four patients, GFR rose by 20 percent or more by the time the EDTA test had fallen to less than 850 µg Pb/day. The rise in GFR was paralleled by increases in effective renal plasma flow (C_{pah}) during EDTA treatment. These findings indicate that chronic lead nephropathy may be reversible by chelation therapy, at least during the pre-azotemic phase of the disease (Wedeen et al., 1979). However, much more information will have to be obtained on the value of long-term, low-dose chelation therapy before this regimen can be widely recommended. There is, at present, no evidence that interstitial nephritis itself is reversed by chelation therapy. It may well be that only functional derangements are corrected and that the improvement in GFR is not accompanied by disappearance of tubulo-interstitial changes in kidney. Chronic volume depletion, for example, might be caused by lead-induced depression of the renin-angiotension-aldosterone system (McAllister et al., 1971) or by direct inhibition of (Na^+, K^+)ATPase-mediated sodium transport (Nechay and Williams, 1977; Nechay and Saunders, 1978a,b,c; Raghavan et al., 1981; Secchi et al., 1973). On the other hand, volume depletion would be expected to produce prerenal azotemia, but this was not evident in these patients. The value of chelation therapy in chronic lead nephropathy once azotemia is established is unknown.

The prevalence of azotemia among lead workers has recently been confirmed in health surveys conducted at industrial sites (Baker et al., 1979; Hammond et al., 1980; Landrigan et al., 1982; Lilis et al., 1979, 1980). Interpretation of these data is, however, hampered by the weak correlation generally found between blood lead levels and chronic lead nephropathy in adults, the absence of matched prospective controls, and the lack of detailed diagnostic information on the workers found to have renal dysfunction. Moreover, blood serum urea nitrogen (BUN) is a relatively poor indicator of renal function because it is sensitive to a variety of physiological variables other than GFR, including protein anabolism, catabolism, and hydration. Several other measures of renal function are more reliable than the BUN, including in order of increasing clinical reliability: serum creatinine, endogenous creatinine clearance, and ^{125}I-iothalamate or inulin clearance. It should be noted that none of these measures of GFR can be considered reliable in the presence of any acute illness such as lead colic or encephalopathy. Elevated BUN in field surveys may, therefore, sometimes represent transient acute functional changes rather than chronic intrinsic renal disease.

The variable susceptibility of the kidneys to the nephrotoxic effects of lead suggests that environmental factors in addition to lead may participate in the expression of renal damage. Industrial workers are often exposed to a variety of toxic materials, some of which, such as cadmium (Buchet et al., 1980), are themselves nephrotoxic. In contrast to cadmium, lead does not increase urinary excretion of beta-2-microglobulins (Batuman et al., 1981; Buchet et al., 1980) or lysozyme (Wedeen et al., 1979) independently of increased low-molecular-weight proteinuria induced by renal failure itself. Multiple interactions between environmental toxins may enhance susceptibility to lead nephrotoxicity. Similarly, nephrotoxicity may be modulated by reductions in 1,25-dihydroxyvitamin D_3, increased 6-betahydroxycortisol production (Saenger et al., 1981, 1982a,b), or immunologic alterations (Gudbrandsson et al., 1981; Koller and Brauner, 1977; Kristensen, 1978; Kristensen and Andersen, 1978). Reductions in dietary intake of calcium, copper, or iron similarly appear to increase susceptibility to lead intoxication (Mahaffey and Michaelson, 1980).

The slowly progressive chronic lead nephropathy resulting from years of relatively low-dose lead absorption (i.e., insufficient to produce symptoms of acute intoxication) observed in adults is strikingly different from the acute lead nephropathy arising from the relatively brief but intense exposure arising from childhood pica. Typical acid-fast intranuclear inclusions are, for example, far less common in the kidneys of adults (Cramer et al., 1974; Wedeen et al., 1975). Although aminoaciduria has been found to be greater in groups of lead workers than in controls (Clarkson and Kench, 1956; Goyer et al., 1972), proximal tubular dysfunction is more difficult to demonstrate in adults with chronic lead nephropathy than in acutely exposed children (Cramer et al., 1974). It should be remembered, however, that children with the Fanconi syndrome have far more severe acute lead intoxication than is usual for workmen on the job. In contrast to the reversible Fanconi syndrome associated with childhood lead poisoning, proximal tubular reabsorptive defects in occupationally exposed adults are uncommon and subtle; clearance measurements are often required to discern impaired tubular reabsorption in chronic lead nephropathy. Hyperuricemia is frequent among lead workers (Albahary et al., 1965; Garrod, 1859; Hong et al., 1980; Landrigan et al., 1982), presumably a consequence of specific lead inhibition of uric acid excretion, increased uric acid production (Emmerson et al., 1971; Granick et al., 1978; Ludwig, 1957), and pre-renal azotemia from volume depletion. The hyperuricemia in adults contrasts with the reduced serum uric acid levels usually associated with the Fanconi syndrome in childhood lead poisoning. Although aminoaciduria and glycosuria are unusual in chronic lead nephropathy, Hong et al. (1980) reported a disproportionate reduction in the maximum reabsorptive rate for glucose compared with para-aminohippurate (PAH) in five of six lead workers they studied. PAH transport has not been consistently altered beyond that expected in renal failure of any etiology (Hong et al., 1980; Wedeen et al., 1975). Biagini et al. (1977) have, however, reported a good negative linear correlation between the one-day EDTA lead-mobilization test and C_{pah} in 11 patients with histologic evidence of lead-induced ultrastructural abnormalities in proximal tubules.

The differences between lead nephropathy in children and adults would not appear to be a consequence of the route of exposure, since a case of pica in an adult (geophagic lead nephropathy) studied by Wedeen et al. (1978) showed the characteristics of chronic rather than acute lead nephropathy. Intranuclear inclusions were absent, and the GFR was reduced out of proportion to the effective renal plasma flow.

12.5.3.4 Lead and Gouty Nephropathy. Renal disease in gout can often be attributed to well-defined pathogenetic mechanisms including urinary tract stones and acute hyperuricemic nephropathy with intratubular uric acid deposition (Bluestone et al., 1977). In the absence of intra- or extra-renal urinary tract obstruction, the frequency, mechanism, and even the existence of a renal disease peculiar to gout remains in question. While some investigators have described "specific" uric acid-induced histopathologic changes in both glomeruli and tubules (Gonick et al., 1965; Sommers and Churg, 1982), rigorously defined controls with comparable degrees of renal failure were not studied simultaneously. Specific histologic changes in the kidneys in gout have not been found by others (Pardo et al., 1968; Bluestone et al., 1977). Glomerulonephritis, vaguely defined "pyelonephritis" (Heptinstall, 1974), or intra- and extra-renal obstruction may have sometimes been confused with the gouty kidney, particularly in earlier studies (Fineberg and Altschul, 1956; Gibson et al., 1980b; Mayne, 1955; McQueen, 1951; Schnitker and Richter, 1936; Talbott and Terplan, 1960; Williamson, 1920).

The histopathology of interstitial nephritis in gout appears to be non-specific and cannot usually be differentiated from that of pyelonephritis, nephrosclerosis, or lead nephropathy on morphologic grounds alone (Barlow and Beilin, 1968; Bluestone et al., 1977; Greenbaum et al., 1961; Heptinstall, 1974; Inglis et al., 1978). Indeed, renal histologic changes in non-gouty hypertensive patients have been reported to be identical to those found in gout patients (Cannon et al., 1966). In these hypertensive patients, serum uric acid levels paralleled the BUN.

Confusion between glomerular and interstitial nephritis can in part be explained by the tendency of proteinuria to increase as renal failure progresses, regardless of the underlying etiology (Batuman et al., 1981). In the absence of overt lead intoxication it may, therefore, be difficult to recognize surreptitious lead absorption as a factor contributing to renal failure in gouty patients. Further, medullary urate deposits, formerly believed to be characteristic of gout (Brown and Mallory, 1950; Mayne, 1955; McQueen, 1951; Fineberg and Altschul, 1956; Talbott and Terplan, 1960), have more recently been reported in end-stage renal disease patients with no history of gout (Cannon et al., 1966; Inglis et al., 1978; Linnane et al., 1981; Ostberg, 1968; Verger et al., 1967). Whether such crystalline deposits contribute to, or are a consequence of, renal damage cannot be determined with confidence. In the presence of severe hyperuricemia (serum uric acid greater than 20 μg/dl), intraluminal crystal deposition may produce acute renal failure because of tubular obstruction associated with grossly visible medullary streaks (Emmerson, 1980). In chronic renal failure without gout or massive hyperuricemia, the functional significance of such medullary deposits is unclear (Linnane et al., 1981). Moreover, medullary microtophi, presumably developing around intraluminal deposits, may extend into the renal interstitium, inducing foreign body reactions with giant cell formation. Such deposits are sometimes overlooked in routine histologic preparations, as they may be dissolved in aqueous fixatives. Their histologic identification requires alcohol fixation and deGalantha staining (Verger et al., 1967). Because of the acid milieu, medullary deposits are usually uric acid, while microtophi developing in the neutral pH of the renal cortex are usually monosodium urate. Both amorphous and needle-like crystals have been demonstrated in kidneys of non-gout and hyperuricemic patients, frequently in association with arteriolonephrosclerosis (Inglis et al., 1978; Cannon et al., 1966; Ostberg, 1968). Urate deposits, therefore, are not only not diagnostic, but may be the result, rather than the cause, of interstitial nephritis. The problem of identifying unique characteristics of the gouty kidney has been further confounded by the coexistence of pyelonephritis, diabetes mellitis, hypertension, and the aging process itself.

Although the outlook for gout patients with renal disease was formerly considered grim (Talbott, 1949; Talbott and Terplan, 1960), more recent long-term follow-up studies suggest a benign course in the absence of renovascular or other supervening disease (Fessel, 1979; Yü and Berger, 1982; Yü, 1982). Over the past four decades the reported incidence of renal disease in gout patients has varied from greater than 25 percent (Fineberg and Altschul, 1956; Hench et al., 1941; Talbott, 1949; Talbott and Terplan, 1960; Wyngaarden, 1958) to less than 2 percent, as observed by Yü (1982) in 707 patients followed from 1970 to 1980. The low incidence of renal disease in some hyperuricemic populations does not support the view that elevated serum uric acid levels of the degree ordinarily encountered in gout patients are harmful to the kidneys (Emmerson, 1980; Fessel, 1979; Ramsay, 1979; Reif et al., 1981). Similarly, the failure of the xanthine oxidase inhibitor, allopurinol, to reverse the course of renal failure in gout patients despite marked reductions in the serum uric acid (Bowie et al., 1967; Levin and Abrahams, 1966; Ogryzlo et al., 1966; Rosenfeld, 1974; Wilson et al., 1967) suggests that renal disease in gout may be due in part to factors other than uric acid. Some studies have, however, suggested a possible slowing of the rate of progression of renal failure in gout by allopurinol (Gibson et al., 1978, 1980a,b; Briney et al., 1975). While the contribution of uric acid to the renal disease of gout remains controversial, the hypothesized deleterious effect of hyperuricemia on the kidney has no bearing on other potential mechanisms of renal damage in these patients.

Although hyperuricemia is universal in patients with renal failure, gout is rare in such patients except when the renal failure is due to lead. Gout occurs in approximately half of the patients with lead nephropathy (Emmerson, 1963, 1973; Ball and Sorensen, 1969; Richet et al., 1965). Moreover, among gout patients in Scotland without known lead exposure, blood lead levels were found to be higher than in non-gouty controls (Campbell et al., 1978). The long association of lead poisoning with gout raises the possibility that lead absorption insufficient to produce overt lead intoxication may, nevertheless, cause gout with slowly progressive renal failure. Garrod (1859), Ball and Sorensen (1969), and Emmerson et al., (1971) demonstrated that lead reduces uric acid excretion, thereby creating the internal milieu in which gout can be expected. The mechanism of hyperuricemia in lead poisoning is, however, unclear. Serum uric acid levels would be expected to rise in association with lead-induced pre-renal azotemia; increased proximal tubule reabsorption of uric acid could result from reduced glomerular filtration rate due to chronic volume depletion. Increased tubular reabsorption of uric acid in lead nephropathy has been suggested by the pyrazinamide suppression test (Emmerson et al., 1971), but interpretation of this procedure was questioned (Holmes and Kelley, 1974). Lead-induced inhibition of tubular secretion of uric acid, therefore, remains another possible mechanism of reduced uric acid excretion. In addition, some investigators have found increased uric acid excretion in saturnine gout patients, thereby raising the possibility that lead increases uric acid production in addition to reducing uric acid excretion (Emmerson et al., 1971; Ludwig, 1957; Granick et al., 1978).

To test the hypothesis that undetected lead absorption may sometimes contribute to renal failure in gout, Batuman et al. (1981) administered the EDTA lead-mobilization test to 44 armed service veterans with gout and assessed their renal function. Individuals currently exposed to lead (e.g., lead workers) were excluded. Collection of urine during the EDTA lead-mobilization test was extended to three days because reduced GFR delays excretion of the lead chelate (Emmerson, 1963). Note that the EDTA test does not appear to be nephrotoxic even for patients with preexisting renal failure (Wedeen et al., 1983). Half of the gout patients had normal renal function and half had renal failure as indicated by serum creatinines over 1.5

mg/dl (mean = 3.0; standard error = 0.4 mg/dl), reflecting approximately 70 percent reduction in renal function. The groups were comparable with regard to age, duration of gout, incidence of hypertension, and history of past lead exposure. The mean (and standard error) blood lead concentration was 26 (± 3) µg/dl in the patients with reduced renal function and 24 (± 3) µg/dl in the gout patients with normal kidney function. The gout patients with renal dysfunction, however, excreted significantly more lead chelate than did those without renal dysfunction (806 ± 90 versus 470 ± 52 µg lead over 3 days). Ten control patients with comparable renal failure excreted 424 ± 72 µg lead during the 3-day EDTA test (2 g i.m.). The non-gout control patients with renal failure had normal lead stores (Emmerson, 1973; Wedeen et al., 1975), indicating that the excessive mobilizable lead in the gout patients with renal failure was not a consequence of reduced renal function _per se_. The source of lead exposure in these armed service veterans could not be determined with confidence. A history of transient occupational exposure and occasional moonshine consumption was common among all the veterans, but the medical histories did not correlate with either the EDTA lead-mobilization test or the presence of renal failure. The relative contributions of airborne lead, industrial sources, and illicit whiskey to the excessive body lead stores demonstrated by the EDTA lead-mobilization test could not, therefore, be determined.

These studies suggest that excessive lead absorption may sometimes be responsible for the gouty kidney in contemporary patients, as appeared to be the case in the past (Wedeen, 1981). Although the EDTA lead-mobilization test cannot prove the absence of other forms of renal disease, a positive EDTA test can indicate that lead may be a contributing cause of renal failure when other known causes are excluded by appropriate diagnostic studies.

12.5.3.5 _Lead and Hypertensive Nephrosclerosis_. Hypertension is another putative complication of excessive lead absorption that has a long and controversial history. In the older literature hypertension was often linked to lead poisoning, frequently in association with renal failure (Beevers et al., 1980; Dingwall-Fordyce and Lane, 1963; Emmerson, 1963; Legge, 1901; Lorimer, 1886; Morgan, 1976; Oliver, 1891; Richet et al., 1966; Vigdortchik, 1935); but a number of other investigators also failed to find such an association (Belknap, 1936; Brieger and Rieders, 1959; Cramer and Dahlberg, 1966; Fouts and Page, 1942; Malcolm, 1971; Mayers, 1947; Ramirez-Cervantes et al., 1978). Much more consistent evidence for associations between lead exposure and hypertension has emerged, however, from numerous recent studies (as discussed in the 1986 Addendum to this document). This includes epidemiological evidence which suggests that hypertension is possibly mediated by lead-induced renal effects. Some of the evidence pointing toward renal involvement is concisely reviewed below.

Among non-occupationally exposed individuals in Scotland, hypertension and serum uric acid levels have been found to correlate with blood lead levels (Beevers et al., 1976). The kidneys of patients with chronic lead nephropathy may show uric acid deposits and the vascular changes of "benign essential hypertension" even in the absence of gout and hypertension (Cramer et al., 1974; Inglis et al., 1978; Morgan, 1976; Wedeen et al., 1975). In a long-term follow-up study of 624 patients with gout, Yü and Berger (1982) reported that while hyperuricemia alone had no deleterious effect on renal function, decreased renal function was more likely to occur in gout patients with hypertension and/or ischemic heart disease than in those with uncomplicated gout.

Hypertension by itself is widely accepted as a cause of renal failure, although the renal sequelae of moderate hypertension appear to be less dramatic than in the past (Kincaid-Smith, 1982). In order to determine if unsuspected excessive body lead stores might contribute to the renal disease of hypertension, 3-day EDTA (2 g i.m.) lead-mobilization tests were performed in hypertensive armed service veterans with and without renal failure (Batuman et al., 1983). A significant increase in mobilizable lead was found in hypertensive subjects with renal disease compared to those without renal disease. Control patients with renal failure again demonstrated normal mobilizable lead, thereby supporting the view that renal failure is not responsible for the excess mobilizable lead in patients with hypertension and renal failure. These findings suggest that patients who would otherwise be deemed to have essential hypertension with nephrosclerosis can be shown to have underlying lead nephropathy by the EDTA lead-mobilization test when other renal causes of hypertension are excluded.

The mechanism whereby lead induces hypertension remains unclear. Although renal disease, particularly at the end-stage, is a recognized cause of hypertension, renal arteriolar histologic changes may precede both hypertension and renal disease (Wedeen et al., 1975). Lead may therefore induce hypertension by direct or indirect effects on the vascular system (see Section 12.9.1 and the Addendum to this document).

Studies of hypertension in moonshine consumers have indicated the presence of hyporeninemic hypoaldosteronism. A blunted plasma renin response to salt depletion has been described in lead poisoned patients; this response can be restored to normal by chelation therapy (McAllister et al., 1971; Gonzalez et al., 1978; Sandstead et al., 1970a). The diminished renin-aldosterone responsiveness found in moonshine drinkers could not, however, be demonstrated in occupationally exposed men with acute lead intoxication (Campbell et al., 1979). Although the impairment of the renin-aldosterone system appears to be independent of renal failure and hypertension, hyporeninemic hypoaldosteronism due to lead might contribute to the hyperkalemia (Morgan, 1976) and the exaggerated natriuresis (Fleischer et al., 1980) of some patients with "benign essential hypertension." Since urinary kallikrein excretion is reduced in lead workers with hypertension, it has been suggested that the decrease in this vasodilator may contribute to lead-induced hypertension (Boscolo et al., 1981). The specificity of kallikrein suppression in the renal and hypertensive manifestions of excessive lead absorption cannot, however, be determined from available data, because lead workers without hypertension and essential hypertensive patients without undue lead absorption also have reduced urinary kallikrein excretion.

12.5.3.6 General Population Studies. Few studies have been performed to evaluate the possible harmful effects of lead on the kidneys in populations without suspected excessive lead absorption from occupational or moonshine exposure.

An epidemiological survey in Scotland of households with water lead concentrations in excess of WHO recommendations (100 µg/l) revealed a close correlation between water lead content and blood lead and serum urea concentrations (Campbell et al., 1977). In 970 households lead concentrations in drinking water ranged from <0.1 to >8.0 mg/l. After clinical and biochemical screening of 283 subjects from 136 of the households with water lead concentrations in excess of 100 µg/l, a subsample of 57 persons with normal blood pressure and elevated serum urea (40 µg/dl) was compared with a control group of 54 persons drawn from the study group with normal blood pressure and normal serum urea. The frequency of renal dysfunction in individuals with elevated blood lead concentratons (>41 µg/dl) was significantly greater than that of age- and sex-matched controls.

Since 62 general practitioners took part in the screening, the subsamples may have come from many different areas; however, it was not indicated if matching was done for place of residence. The authors found a significantly larger number of high blood lead concentrations among the persons with elevated serum urea and claimed that elevated water lead concentration was associated with renal insufficiency as reflected by raised serum urea concentrations. This conclusion is difficult to accept since serum urea is not the method of choice for evaluating renal function. Despite reservations concerning use of the BUN for assessing renal function (due to transient fluctuations), these findings are consistent with the view that excessive lead absorption from household water causes renal dysfunction. However, the authors used unusual statistical methods and could not exclude the reverse causal relationship, i.e., that renal failure had caused elevated blood lead levels in their study group. A carefully matched control population of azotemic individuals from low lead households would have been helpful for this purpose. A more convincing finding in another subsample was a strong association between hyperuricemia and blood lead level. This was also interpreted as a sign of renal insufficiency, but it may have represented a direct effect of lead on uric acid production or renal excretion.

Campbell et al. (1977) also found a statistically significant correlation between blood lead concentration and hypertension. Tap-water lead did not, however, correlate with blood lead among the hypertensive group, thus suggesting that other environmental sources of lead may account for the presence of high blood lead concentrations among hypertensive persons in Scotland (Beevers et al., 1976, 1980).

12.5.4 Mortality Data

Cooper and Gaffey (1975) analyzed mortality data available from 1267 death certificates for 7032 lead workers who had been hired by 16 smelting or battery plants between 1900 and 1969. Standardized mortality ratios revealed an excess of observed over predicted deaths from "other hypertensive disease" and "chronic nephritis and other renal sclerosis." The authors concluded that "high levels of lead absorption such as occurred in many of the workers in this series, can be associated with chronic renal disease." In an extension of this mortality study covering the period 1971-1975 (Cooper, 1981), the excess of deaths from "other hypertensive disease" and "chronic nephritis" was no longer evident. In the follow-up study, deaths from major cardiovascular and renal diseases were "slightly higher than expected," but did not reach statistical significance (Cooper, 1981). Cooper (1984) recently reexamined data for a more rigorously selected subset of the same population (6819 workers versus 7032 originally) for the period 1947-1980. He found significantly greater than expected mortality for both battery plant and lead smelter workers. These excess deaths appeared to result primarily from malignant neoplasms (but not renal malignancies), chronic renal disease, and "ill-defined" causes. Chronic renal disease reflected two general classifications: "other hypertensive disease" and "chronic and unspecified nephritis." Most of these deaths occurred prior to 1971, which accounts for the lack of such findings in Cooper's (1981) analysis of 1971-1975 data. Cooper (1984) noted that, although battery plant workers showed the greatest excess of deaths from these causes, they did not show significantly elevated standardized mortality ratios for hypertensive heart disease or stroke. Despite the lack of excess of renal carcinoma in Cooper's analyses, kidney tumors have been found in lead-poisoned experimental animals (see Section 12.7) and in at least two cases of occupationally exposed workers (Baker et al., 1980; Lilis, 1981). Selevan et al. (1984) have also noted an increased, but not statistically significant, incidence of renal cancer in a group of lead smelter workers.

In a more limited study of 241 Australian smelter employees who were diagnosed as lead poisoned between 1928 and 1959 by a government medical board, 140 deaths were identified between 1930 and 1977 (McMichael and Johnson, 1982). Standard proportional mortality rates of the lead-exposed workers compared with 695 non-lead-exposed employees revealed an overall threefold excess in deaths due to chronic nephritis and a twofold excess in deaths due to cerebral hemorrhage in the lead-exposed workers. Over the 47 years of this retrospective study, the number of deaths from chronic nephritis decreased from an initial level of 36 percent to 4.6 percent among the lead-exposed workers, compared with a drop from 8.7 percent to 2.2 percent among controls. From 1965 to 1977 the age-standardized mortality rates from chronic nephritis were the same for the lead-worker and control groups, although both rates were higher than the proportional mortality rate for the general population of Australian males. The latter observation indicated that the excessive deaths from chronic nephritis among lead-poisoned workers at the smelter had declined in recent decades.

Despite substantial evidence that lead produces interstitial nephritis in adults, the impact of chronic lead nephropathy on the general population is unknown. The diagnosis of lead nephropathy is rarely made in dialysis patients in the United States. The absence of the diagnosis does not, however, provide evidence for the absence of the disease. Advanced renal failure is usually encountered only many years after excessive lead exposure. Moreover, acute intoxication may never have occurred, and neither heme enzyme abnormalities nor elevated blood lead levels may be present at the time renal failure becomes apparent. The causal relationship between lead absorption and renal disease may therefore not be evident. It is likely that such cases of lead nephropathy have previously been included among other diagnostic categories such as pyelonephritis, interstitial nephritis, gouty nephropathy, and hypertensive nephrosclerosis. Increasing proteinuria as lead nephropathy progresses may also cause confusion with primary glomerulonephritis. It should also be noted that the End Stage Renal Disease Program (U.S. Health Care Financing Administration, 1982) does not even include the diagnosis of lead nephropathy in its reporting statistics, regardless of whether the diagnosis is recognized by the attending nephrologist.

12.5.5 Experimental Animal Studies of the Pathophysiology of Lead Nephropathy

Laboratory studies of experimental animals have helped clarify a number of the mechanisms underlying lead-induced nephropathy in humans. The following discussion will center on the renal uptake and intracellular binding of lead, morphological alterations, various functional changes, and biochemical effects associated with the renal toxicity of lead.

12.5.5.1 Lead Uptake by the Kidney. Lead uptake by the kidney has been studied *in vivo* and *in vitro* using slices of renal tissue. Vander et al. (1977) performed renal clearance studies in dogs two hours after a single i.v. dose of 0.1 or 0.5 mg lead acetate containing 1-3 mCi of ^{203}Pb or 1 hour after continous i.v. infusion of 0.1-0.15 mg/kg-hour. These investigators reported that 43-44 percent of the plasma lead was ultrafiltrable, with kidney reabsorption values of 89-94 percent for the ultrafiltrable fraction. A subsequent stop-flow analysis investigation by Victery et al. (1979a), using dogs given a single i.v. dose of lead acetate at 0.2 or 10.0 mg/kg, showed both proximal and distal tubular reabsorption sites for lead. Distal reabsorption was not linked to sodium chloride or calcium transport pathways. Proximal tubule reabsorption was demonstrated in all animals tested during citrate or bicarbonate infusion. Another experiment by Victery et al. (1979b) examined the influence of acid-base status on renal accumulation and excretion of lead in dogs given 0.5-50 μg/kg hour as an infusion or in rats given access to drinking water containing 500 ppm lead for 2-3 months. They showed that alkalosis increased lead entry into tubule cells via both luminal and basolateral membranes, with a resultant increase in both renal tissue accumulation and urinary excretion of lead. Similarly, acutely induced alkalosis increased lead excretion in rats previously exposed through their drinking water. The authors concluded that earlier results from acute exposure experiments on the renal handling of lead (Vander et al., 1977) were at least qualitatively similar to results of the chronic exposure experiments and that rats were an acceptable model for investigating the effects of alkalosis on the excretion of lead following chronic exposure.

In vitro studies (Vander et al., 1979) using slices of rabbit kidney incubated with ^{203}Pb acetate at lead concentrations of 0.1 or 1.0 μM over 180-minute time intervals showed that a steady-state uptake of ^{203}Pb by slices (ratio of slice to medium uptake in the range of 10-42) was reached after 90 minutes and that lead could enter the slices as a free ion. Tissue slice uptake was reduced by a number of metabolic inhibitors, thus suggesting a possible active transport mechanism. Tin (Sn IV) markedly reduced ^{203}Pb uptake into the slices but did not affect lead efflux or para-aminohippurate accumulation. This finding raises the possibility that Pb and Sn (IV) compete for a common carrier. Subsequent studies also using rabbit kidney slices (Vander and Johnson, 1981) showed that co-transport of ^{203}Pb into the slices in the presence of organic anions such as cysteine, citrate, glutathione, histidine, or serum ultrafiltrate was relatively small compared with uptake due to ionic lead.

In summary, it is clear from the above *in vivo* and *in vitro* studies on several different animal species that renal accumulation of lead is an efficient process that occurs in both proximal and distal portions of the nephron and at both luminal and basolateral membranes. The transmembrane movement of lead appears to be mediated by an uptake process that is subject to inhibition by several metabolic inhibitors and the acid-base status of the organism.

12.5.5.2 Intracellular Binding of Lead in the Kidney. The bioavailability of lead inside renal tubule cells under low or ^{203}Pb-tracer exposure conditions is mediated in part by binding to several high-affinity cytosolic binding proteins (Oskarsson et al., 1982; Mistry et al., 1982) and, at higher exposure conditions, by the formation of cytoplasmic and intranuclear inclusion bodies (Goyer et al., 1970a). These inclusion bodies have been shown by both cell fractionation (Goyer et al., 1970a) and X-ray microanalysis (Fowler et al., 1980) to contain the highest intracellular concentrations of lead. Saturation analysis of the renal

63,000 dalton (63K) cytosolic binding protein has shown that it possesses an approximate dissociation constant (K_d) of 10^{-8} M (Mistry et al., 1982). These data quantify the high-affinity nature of this protein for lead and explain the previously reported finding (Oskarsson et al., 1982) that this protein constitutes a major intracellular lead-binding site in the kidney cytosol. Biochemical studies on the protein components of isolated rat kidney intranuclear inclusion bodies have shown that the main component has an approximate molecular weight of 27K (Moore et al., 1973) or 32K (Shelton and Egle, 1982) and that it is rich in two dicarboxylic amino acids, glutamate and aspartate (Moore et al., 1973). The isoelectric point of the main nuclear inclusion body protein has been reported to be pI = 6.3 and appeared from two-dimensional gel analysis to be unique to nuclei of lead-injected rats (Shelton and Egle, 1982). The importance of the inclusion bodies resides with the suggestion (Goyer et al., 1970a; Moore et al., 1973; Goyer and Rhyne, 1973) that, since these structures contain the highest intracellular concentrations of lead in the kidney proximal tubule and hence account for much of the total cellular lead burden, they sequester lead to some degree away from sensitive renal organelles or metabolic (e.g., heme biosynthetic) pathways until their capacity is exceeded. The same argument would apply to the high-affinity cytosolic lead-binding proteins at lead exposure levels below those that cause formation of inclusion bodies. It is currently unclear whether lead-binding to these proteins is an initial step in the formation of the cytoplasmic or nuclear inclusion bodies (Oskarsson et al., 1982).

12.5.5.3 Pathological Features of Lead Nephropathy. The main morphological effects of lead in the kidney are manifested in renal proximal tubule cells and interstitial spaces between the tubules. A summary of morphological findings from some recent studies involving a number of animal species is given in Table 12-11. In all but one of these studies, formation of intranuclear inclusion bodies is a common pathognomic feature for all species examined. In addition, proximal tubule cell cytomegaly and swollen mitochondria with increased numbers of lysosomes were also observed in two of the chronic exposure studies (Fowler et al., 1980; Spit et al., 1981). Another feature reported in three of these studies (Hass et al., 1964; White, 1977; Fowler et al., 1980) was the primary localization of morphological changes in the straight (S_3) segments of the proximal tubule, thereby indicating that not all cell types of the kidney are equally involved in the toxicity of lead to this organ. Interstitial fibrosis has also been reported in rabbits (Hass et al., 1964) given diets containing 0.5 percent lead acetate for up to 55 weeks and in rats (Goyer, 1971) given drinking water containing lead acetate for 9 weeks.

12.5.5.4 Functional Studies

12.5.5.4.1 Renal blood flow and glomerular filtration rate. Studies by Aviv et al. (1980) concerning the impact of lead on renal function as assessed by renal blood flow (RBF) and glomerular filtration rate (GFR) have reported significant ($p < 0.01$) reductions in both of

TABLE 12-11. MORPHOLOGICAL FEATURES OF LEAD NEPHROPATHY IN VARIOUS SPECIES

		Morphological findings*				
Species	Pb dose regimen	Nuclear inclusions	Increased mitochondrial swelling	Increased lysosomes	Interstitial fibrosis	Reference
Rabbit	0.5% Pb acetate in diet for up to 55 weeks	+	ND	ND	+	Hass et al. (1964)
Rat	1% Pb in drinking water for 9 weeks	+	+	ND	+	Goyer (1971)
Dog	50 μg/kg by gavage for 5 weeks	+	ND	ND	ND	White (1977)
Monkey	0, 1.5, 6.0, 15 μg/day in ad lib. drinking water 6 days/week for 9 months	+	ND	ND	ND	Colle et al. (1980)
Rat	0, 0.5, 5, 25, 50, 250 ppm in ad lib. drinking water	+	+	-	-	Fowler et al. (1980)
Rabbit	0, 0.25, 0.50 μg/kg by subcutaneous injection 3 days/wk for 14 weeks	-	-	+	-	Spit et al. (1981)
Ringed dove	100 μg/ml in ad lib. drinking water	+	+	-	-	Kendall et al. (1981)

*ND = Not determined; + = positive finding; - = negative finding.

these parameters in rats at 3 and 16 weeks after termination of exposure to 1 percent lead acetate in drinking water. A statistically significant ($p < 0.05$) reduction of GFR has also been recently described in dogs 2.5-4 hours after a single i.v. dose of lead at 3.0 mg/kg (Victery et al., 1981). In contrast, studies by others (Johnson and Kleinman, 1979; Hammond et al., 1982) were not able to demonstrate a reduction in GFR or RBF using the rat as a model. The reasons behind these reported differences are currently unclear but may be related to differences in experimental design, age of subjects, or other variables.

12.5.5.4.2 Tubular function. Exposure to lead has also been reported to produce tubular dysfunction (Studnitz and Haeger-Aronsen, 1962; Goyer, 1971; Mouw et al., 1978; Suketa et al., 1979; Victery et al., 1981, 1982a,b, 1983). An early study (Studnitz and Haeger-Aronsen, 1962) reported aminoaciduria in rabbits given a single dose of lead at 125 mg/kg, with urine collected over a 15-hour period. Goyer et al. (1970b) described aminoaciduria in rats following exposure to 1 percent lead acetate in the diet for 10 weeks. Wapnir et al. (1979) confirmed a mild hyperaminoaciduria in rats injected with lead at 20 mg/kg five times a week for six weeks but found no changes in urinary excretion of phosphate or glucose.

Other studies (Mouw et al., 1978; Suketa et al., 1979; Victery et al., 1981, 1982a,b, 1983) have focused attention on increased urinary excretion of electrolytes. Mouw et al. (1978) reported increased urinary excretion of sodium, potassium, calcium, and water in dogs given a single intravenous injection of lead at 0.6 or 3.0 mg/kg over a 4-hour period. This effect occurred despite a constant GFR, which indicates decreased tubular reabsorption of these substances. Suketa et al. (1979) treated rats with a single oral dose of lead at 0, 5, 50, or 200 mg/kg and killed the animals at 0, 6, 12, or 24 hours after treatment. A dose-related increase in urinary sodium, potassium, and water was observed over time. Victery et al. (1981, 1982a,b, 1983) studied zinc excretion in dogs over a 4-hour period following an intravenous injection of lead at 0.3 or 3.0 mg/kg. These investigators reported maximal increases in zinc excretion of 140 ng/min at the 0.3 mg/kg dose and 300 ng/min at the 3.0 mg/kg dose at the end of the 4-hour period. In contrast, studies by Mouw et al. (1978) showed no changes in urinary excretion of sodium or potassium. Urinary protein and magnesium excretion were also unchanged.

The results of the above studies indicate that acute or chronic lead treatment is capable of producing tubular dysfunction in various mammalian species, as manifested by increased urinary excretion of amino acid nitrogen, water, and some ions such as Zn^{2+}, Ca^{2+}, Na^{+}, and K^{+}.

12.5.6 Experimental Studies of the Biochemical Aspects of Lead Nephrotoxicity

12.5.6.1 Membrane Marker Enzymes and Transport Functions. The biochemical effects of lead in the kidney appear to be preferentially localized in the cell membranes and mitochondrial and nuclear compartments following either acute or chronic lead exposure regimens.

Oral exposure of rats to lead acetate in the diet at concentrations of 1-2 percent for 10-40 weeks was found to produce no significant changes in the water content of renal slices or in the accumulation of para-aminohippurate or tetraethyl-ammonium. However, tissue glucose synthesis at 40 weeks and pyruvate metabolism were both significantly ($p < 0.05$) reduced (Hirsch, 1973).

Wapnir et al. (1979) examined biochemical effects in kidneys of rats injected with lead acetate (20 mg/kg) five days per week for six weeks. They observed a significant ($p < 0.05$) reduction in renal alkaline phosphatase activity and an increase in (Mg^{2+})-ATPase, but no significant changes in (Na^+,K^+)-ATPase, glucose-6-phosphatase, fructose 1-6 diphosphatase, tryptophan hydroxylase, or succinic dehydrogenase. These findings indicated that preferential effects occurred only in marker enzymes localized in the brush border membrane and mitochondrial inner membrane. Suketa et al. (1979) reported marked (50-90 percent) decreases in renal (Na^+,K^+)-ATPase at 6-24 hours following a single oral administration of lead acetate at a dose of 200 mg/kg. A later study (Suketa et al., 1981) using this regimen showed marked decreases in renal (Na^+, K^+)-ATPase but no significant changes in (Mg^{2+})-ATPase after 24 hours, thus indicating inhibition of a cell membrane marker enzyme prior to changes in a mitochondrial marker enzyme.

12.5.6.2 Mitochondrial Respiration/Energy-Linked Transformation. Effects of lead on renal mitochondrial structure and function have been studied by a number of investigators (Goyer, 1968; Goyer and Krall, 1969a,b; Fowler et al., 1980, 1981a,b). Examination of proximal tubule cells of rats exposed to drinking water containing 0.5-1.0 percent lead acetate for 10 weeks (Goyer, 1968; Goyer and Krall, 1969a,b) or 250 ppm lead acetate for 9 months (Fowler et al., 1980) has shown swollen proximal tubule cell mitochondria *in situ*. Common biochemical findings in these studies were decreases in respiratory control ratios (RCR) and inhibition of state-3 respiration, which was most marked for NAD-linked substrates such as pyruvate/malate. Goyer and Krall (1969a,b) found these respiratory effects to be associated with a decreased capacity of mitochondria to undergo energy-linked structural transformation.

In vitro studies (Garcia-Cañero et al., 1981) using 10^{-4} M lead demonstrated decreased renal mitochondrial membrane transport of pyruvate or glutamate associated with decreased respiration for these two substrates. Other *in vitro* studies (Fowler et al., 1981a,b) have shown decreased renal mitochondrial membrane energization as measured by the fluorescent probes 1-anilino,-8 napthalenesulfonic acid (ANS) or ethidium bromide following exposure to lead acetate at concentrations of 10^{-5}-10^{-3} M lead. High amplitude mitochondrial swelling was also observed by light scattering.

The results of the above studies indicate that lead produces mitochondrial swelling both *in situ* and *in vitro*, associated with a decrease in respiratory function that is most marked for RCR and state-3 respiration values. The structural and respiratory changes appear to be linked to lead-induced alteration of mitochondrial membrane energization.

12.5.6.3 Renal Heme Biosynthesis. There are several reports concerning the effects of lead on renal heme biosynthesis following acute or chronic exposure (see Table 12-12). Silbergeld et al. (1982) injected rats with lead at 10 µmol/kg per day for three days and examined effects on several tissues including kidney. These investigators found an increase in δ-aminolevulinic acid synthetase (ALA-S) following acute injection and no change following chronic exposure (first indirectly via their dams' drinking water containing lead at 10 mg/ml until 30 days of age and then directly via this drinking water until 40-60 days of age). Renal tissue content of δ-aminolevulinic acid (ALA) was increased in both acutely and chronically exposed rats. Renal δ-aminolevulinic acid dehydrase (ALA-D) was found to be inhibited in both acute and chronic treatment groups. Gibson and Goldberg (1970) injected rabbits s.c. with lead acetate at doses of 0, 10, 30, 150, or 200 mg/week for up to 24 weeks. The mitochondrial enzyme ALA-S in kidney was found to show no measurable differences from control levels. Renal ALA-D, which is found in the cytosol fraction, showed no differences from control levels when glutathione was present but was significantly reduced ($p < 0.05$) to 50 percent of control values for the pooled lead-treated groups when glutathione was absent. Mitochondrial heme synthetase (ferrochelatase) was not significantly decreased in lead-treated versus control rabbits, but this enzyme in the kidney was inhibited by 72 and 94 percent at lead-acetate concentrations of 10^{-4} and 10^{-3} M lead, respectively. Accumulation (12-15 fold) of both ALA and porphobilinogen (PBG) was also observed in kidney tissue of lead-treated rabbits relative to controls. Zawirska and Medraś (1972) injected rats with lead acetate at a dose of 3 mg/day for up to 60 days and noted a similar renal tissue accumulation of uroporphyrin, coproporphyrin, and protoporphyrin. A study by Fowler et al. (1980) using rats exposed through 9 months of age to 50 or 250 ppm lead acetate in drinking water showed significant inhibition of the mitochondrial enzymes ALA-S and ferrochelatase but no change in the activity of the cytosolic enzyme ALA-D. Similar findings have been reported for ALA-D following acute i.p. injection of lead acetate at doses of 5-100 mg/kg at 16 hours prior to sacrifice (Woods and Fowler, 1982). In the latter two studies, reduced glutathione was present in the assay mixture.

To summarize the above studies, the pattern of alteration of renal heme biosynthesis by lead is somewhat different from that usually observed with this agent in other tissues (see Section 12.3). In general, renal ALA-D does not seem to be inhibited much by lead except under conditions of high-level exposure (Table 12-12). Such a finding could result from the presence of the recently described high affinity cytosolic lead-binding proteins (Oskarsson et al., 1982; Mistry et al., 1982) in the kidney and/or the formation of lead-containing intranuclear inclusion bodies in this tissue (Goyer, 1971; Fowler et al., 1980), which would

TABLE 12-12. EFFECTS OF LEAD EXPOSURE ON ASPECTS OF RENAL HEME BIOSYNTHESIS

		Parameter Affected[a]				
Species	Pb dose regimen[b]	ALA-S	ALA-D	FC[c]	Renal tissue porphyrins	Reference
Rabbit	10, 30, 150, 200 mg/kg per wk (s.c.)	NC	±↓	NC	↑ ALA, PBG (12-15x)	Gibson and Goldberg (1970)
Rat	3 mg/day (s.c.)	ND	ND	ND	↑ uro-, copro-, protoporphyrins	Zawirska and Medraś (1972)
Rat	10, 100, 1000, 5000 ppm in d.w. for 3 wks	ND	↓	ND	↑ at 1000 and 5000 ppm; ↑ ALA-urine	Buchet et al. (1976)
Rat (dams)	10 ppm in d.w. during: 3 wks before mating 3 wks of pregnancy 3 wks after delivery	ND	NC	ND	NC	Hubermont et al. (1976)
(newborns)		ND	NC	ND	↑	
Rat (dams)	100 ppm in d.w. for 3 wks	ND	NC	ND	NC	Roels et al. (1977)
(sucklings)		ND	NC	ND	↑	
Rat	0.5, 5, 25, 50, 250 ppm in d.w. for 9 months	↓	NC	↓	ND	Fowler et al. (1980)
Rat	5, 25, 50, 100 mg/kg (i.p.) 16 hrs prior to sacrifice	ND	NC	ND	ND	Woods and Fowler (1982)
Rat	10 μmol/kg/(i.p.) for 3 days	↑	↓	ND	↑ ALA	Silbergeld et al. (1982)
	10 mg/ml in d.w. for 10-30 days	NC	↓	ND	↑ ALA	

[a] ↑ = increased; ↓ = decreased; ± = effect depends on conditions of assay; NC = no change relative to controls; ND = not determined.

[b] s.c. - subcutaneous; i.p. - intraperitoneal; d.w. - drinking water.

[c] FC - ferrochelatase.

sequester most of the intracellular lead away from other organelle compartments until the capacity of these mechanisms is exceeded. Based on the observations of Gibson and Goldberg (1970), tissue or assay concentrations of glutathione may also be of importance to the effects of lead on this enzyme. The observed lack of ALA-S induction in kidney mitochondria reported in the above studies may have been caused by decreased mitochondrial protein synthesis capacity or, as previously suggested (Fowler et al., 1980), by overwhelming inhibition of this enzyme by lead, such that any inductive effects were not measurable. Further research is needed to resolve these questions.

12.5.6.4 Alteration of Renal Nucleic Acid/Protein Synthesis. A number of studies have shown marked increases in renal nucleic acid or protein synthesis following acute or chronic exposure to lead acetate. One study (Choie and Richter, 1972a) conducted on rats given a single intraperitoneal injection of lead acetate showed an increase in ^{3}H-thymidine incorporation. A subsequent study (Choie and Richter, 1972b) involved rats given intraperitoneal injections of 1-7 mg lead once per week over a 6-month period. Autoradiography of ^{3}H-thymidine incorporation into tubule cell nuclei showed a 15-fold increase in proliferative activity in the lead-treated rats relative to controls. The proliferative response involved cells both with and without intranuclear inclusions. Follow-up autoradiographic studies in rats given three intraperitoneal injections of lead acetate (0.05 mg/kg) 48 hours apart showed a 40-fold increase in ^{3}H-thymidine incorporation 20 hours after the first lead dose and 6 hours after the second and third doses.

Choie and Richter (1974a) also studied mice given a single intracardiac injection of lead (5 μg/g) and demonstrated a 45-fold maximal increase in DNA synthesis in proximal tubule cells as judged by ^{3}H-thymidine autoradiography 33 hours later. This increase in DNA synthesis was preceded by a general increase in both RNA and protein synthesis (Choie and Richter, 1974b). The above findings were essentially confirmed with respect to lead-induced increases in nucleic acid synthesis by Cihák and Seifertová (1976), who found a 13-fold increase in ^{3}H-thymidine incorporation into kidney nuclei of mice 4 hours after an intracardiac injection (5 μg/g) of lead acetate. This finding was associated with a 34-fold increase in the mitotic index but no change in the activities of thymidine kinase or thymide monophosphate kinase. Stevenson et al. (1977) have also reported a 2-fold increase in ^{3}H-thymidine or ^{14}C-orotic acid incorporation into kidney DNA or RNA of rats given a single intraperitoneal injection of lead chloride three days earlier.

The above studies clearly demonstrate that acute or chronic administration of lead by injection stimulates renal nucleic acid and protein synthesis in kidneys of rats and mice. The relationship between this proliferative response and formation of intranuclear inclusion bodies is currently unknown; nor is the basic mechanism underlying this response and the formation of renal adenomas in rats and mice following chronic lead exposure understood.

12.5.6.5 Lead Effects on the Renin-Angiotension System. A study by Mouw et al. (1978) used dogs given a single intravenous injection of lead acetate at doses of 0.6 or 3.0 mg/kg and observed over a 4-hour period. Subjects showed a small but significant decrease in plasma renin activity (PRA) at 1 hour, followed by a large and significant ($p < 0.05$) increase from 2.5 to 4.0 hours. Follow-up work (Goldman et al., 1981) using dogs given a single intravenous injection of lead acetate at 3.0 mg/kg showed changes in the renin-angiotensin system over a 3-hour period. The data demonstrated an increase in PRA, but increased renin secretion occurred in only three of nine animals. Hepatic extraction of renin was virtually eliminated in all animals, thus providing an explanation for the increased blood levels of renin. Despite the large observed increases in PRA, blood levels of angiotensin II (AII) did not increase after lead treatment. This suggests that lead inhibited the AII converting enzyme.

Exposure of rats to drinking water containing 0.5 mg Pb/ml for three weeks to five months (Fleischer et al., 1980) produced an elevation of PRA after six weeks of exposure in those rats on a sodium-free diet. No change in plasma renin substrate (PRS) was observed. At five months, PRA was significantly higher in the lead-treated group on a 1-percent sodium chloride diet, but the previous difference in renin levels between animals on an extremely low-sodium (1 meq) versus 1-percent sodium diet had disappeared. The lead-treated animals had a reduced ability to decrease sodium excretion following removal of sodium from the diet.

Victery et al. (1982a) exposed rats to lead *in utero* and to drinking water solution containing 0, 100, or 500 ppm lead as lead acetate for six months. Male rats on the 100 ppm lead dose became significantly hypertensive at 3.5 months and remained in that state until termination of the experiment at six months. All female rats remained normotensive as did males at the 500-ppm dose level. PRA was found to be significantly reduced in the 100-ppm treatment males and normal in the 500-ppm treatment groups of both males and females. Dose-dependent decreases in AII/PRA ratios and renal renin content were also observed. Pulmonary AII converting enzyme was not significantly altered. It was concluded that, since the observed hypertension in the 100-ppm group of males was actually associated with reduction of PRA and AII, the renin-angiotensin system was probably not directly involved in this effect.

Webb et al. (1981) examined the vascular responsiveness of helical strips of tail arteries in rats exposed to drinking water containing 100 ppm lead for seven months. These investigators found that the mild hypertension associated with this regimen was associated with increased vascular responsiveness to α-adrenergic agonists.

Male rats exposed to lead *in utero* and prior to weaning indirectly by their dams' drinking water containing 0, 5, or 25 ppm lead as lead acetate, followed by direct exposure at the same levels for five months (Victery et al., 1982b), showed no change in systolic blood pressure. Rats exposed to the 25 ppm dose showed a significant ($p < 0.05$) decrease in basal PRA. Stimulation of renin release by administration of polyethylene glycol showed a significant increase in PRA but low AII values. These yielded a significant ($p < 0.001$) decrease in the AII/PRA ratio. Basal renal renin concentrations were found to be significantly reduced in both the 5 ppm ($p < 0.05$) and 25 ppm ($p < 0.01$) dose groups relative to controls.

Victery et al. (1983) exposed rats in utero to lead by maternal administration of 0, 5, 25, 100, or 500 ppm lead as lead acetate. The animals were continued on their respective dose levels through one month of age. All exposure groups had PRA values significantly ($p < 0.05$) elevated relative to controls. Renal renin concentration was found to be similar to controls in the 5 and 25 ppm groups but significantly increased ($p < 0.05$) in the 100 and 500 ppm groups. The plasma AII/PRA ratio was similar to controls in the 100 ppm group but was significantly reduced ($p < 0.05$) in the 500 ppm group.

It appears from the above studies that lead exposure at even low dose levels is capable of producing marked changes in the renin-angiotension system and that the direction and magnitude of these changes is mediated by a number of factors, including dose level, age, and sex of the species tested, as well as dietary sodium content. Lead also appears capable of directly altering vascular responsiveness to α-adrenergic agents. The mild hypertension observed with chronic low-level lead exposure appears to stem in part from this effect and not from changes in the renin-angiotensin system. (See also Section 12.9.1 and the Addendum to this document for a discussion of other work on the hypertensive effects of lead.)

12.5.6.6 Lead Effects on Uric Acid Metabolism. A report by Mahaffey et al. (1981) on rats exposed concurrently to lead, cadmium, and arsenic alone or in combination found significantly ($p < 0.05$) increased serum concentrations of uric acid in the lead-only group. While the biochemical mechanism of this effect is not clear, these data support certain observations in humans concerning hyperuricemia as a result of lead exposure (see Section 12.5.3) and, also, confirm an earlier report by Goyer (1971) showing increased serum uric acid concentration in rats exposed to 1 percent lead acetate in drinking water for 84 weeks.

12.5.6.7 Lead Effects on Kidney Vitamin D Metabolism. Smith et al. (1981) fed rats vitamin D-deficient diets containing either low or normal calcium or phosphate for two weeks. The animals were subsequently given the same diets supplemented with 0.82 percent lead as lead acetate. Ingestion of lead at this dose level significantly reduced plasma levels of 1,25 dihydrocholecalciferol in cholecalciferol-treated rats and in rats fed either a low phosphorous or low calcium diet while it had no effect in rats fed either a high calcium or normal phosphorous diet. These data suggest decreased production of 1,25-dihydrocholecalciferol in the kidney in response to lead exposure in concert with dietary deficiencies. These and other data concerning lead effects on Vitamin-D metabolism were earlier discussed in detail in Section 12.3.

12.5.7 General Summary: Comparison of Lead's Effects on Kidneys in Humans and Animal Models

It has been known for more than a century that kidney disease can result from lead poisoning. Identifying the contributing causes and mechanisms of lead-induced nephropathy has been difficult, however, in part because of the complexities of human exposure to lead and other nephrotoxic agents. Nevertheless, it is possible to estimate at least roughly the range of lead exposure associated with detectable renal dysfunction in both human adults and children. Numerous studies of occupationally exposed workers have provided evidence for lead-induced chronic nephropathy being associated with blood lead levels ranging from 40 to more than 100 µg/dl, and some are suggestive of renal effects possibly occurring even at levels as low as 30 µg/dl. In children, the relatively sparse evidence available points to the manifestation of renal dysfunction only at quite high blood lead levels (usually exceeding 120 µg/dl). The current lack of evidence for renal dysfunction at lower blood lead levels in children may simply reflect the greater clinical concern with neurotoxic effects of lead intoxication in children. The persistence of lead-induced renal dysfunction in children also remains to be more fully investigated, although a few studies indicate that children diagnosed as being acutely lead poisoned experience lead nephropathy effects lasting throughout adulthood.

Parallel results from experimental animal studies reinforce the findings in humans and help illuminate the mechanisms underlying such effects. For example, a number of transient effects in human and animal renal function are consistent with experimental findings of reversible lesions such as nuclear inclusion bodies, cytomegaly, swollen mitochondria, and increased numbers of iron-containing lysosomes in proximal tubule cells. Irreversible lesions such as interstitial fibrosis are also well documented in both humans and animals following chronic exposure to high doses of lead. Functional renal changes observed in humans have also been confirmed in animal model systems with respect to increased excretion of amino acids and elevated serum urea nitrogen and uric acid concentrations. The inhibitory effects of lead exposure on renal blood flow and glomerular filtration rate are currently less clear in experimental model systems; further research is needed to clarify the effects of lead on these functional parameters in animals. Similarly, while lead-induced perturbation of the renin-angiotensin system has been demonstrated in experimental animal models, further research is needed to clarify the exact relationships among lead exposure (particularly chronic low-level exposure), alteration of the renin-angiotensin system, and hypertension in both humans and animals.

On the biochemical level, it appears that lead exposure produces changes at a number of sites. Inhibition of membrane marker enzymes, decreased mitochondrial respiratory function/cellular energy production, inhibition of renal heme biosynthesis, and altered nucleic acid synthesis are the most marked changes to have been reported. The extent to which these mitochondrial alterations occur is probably mediated in part by the intracellular bioavailability of lead, which is determined by its binding to high affinity kidney cytosolic proteins and deposition within intranuclear inclusion bodies.

Among the questions remaining to be answered more definitively about the effects of lead on the kidneys is the lowest blood lead level at which renal effects occur. In this regard it should be noted that recent studies in humans have indicated that the EDTA lead-mobilization test is the most reliable technique for detecting persons at risk for chronic nephropathy; blood lead measurements are a less satisfactory indicator because they may not accurately reflect cumulative absorption some time after exposure to lead has terminated. Other questions include the following: Can a distinctive lead-induced renal lesion be identified either in functional or histologic terms? What biologic measurements are most reliable for the prediction of lead-induced nephropathy? What is the incidence of lead nephropathy in the general population as well as among specifically defined subgroups with varying exposure? What is the natural history of treated and untreated lead nephropathy? What is the mechanism of lead-induced hypertension and renal injury? What are the contributions of environmental and genetic factors to the appearance of renal injury due to lead? Conversely, the most difficult question of all may well be to determine the contribution of low levels of lead exposure to renal disease of non-lead etiologies.

12.6 EFFECTS OF LEAD ON REPRODUCTION AND DEVELOPMENT

Studies of humans and animals indicate that lead may exert gametotoxic, embryotoxic, and teratogenic effects that could influence the survival and development of the fetus and newborn. It appears that prenatal viability and development may also be indirectly affected by lead through its effects on the health of the expectant mother. The vulnerability of the conceptus to such effects has contributed to concern that the unborn may constitute a group at risk for the effects of lead on health. Also, certain information regarding male reproductive functions has led to concern regarding the impact of lead on men.

12.6.1 Human Studies

12.6.1.1 Historical Evidence. Findings suggesting that lead exerts adverse effects on human reproductive functions have existed in the literature since before the turn of the century. For example, Paul (1860) observed that severely lead-poisoned pregnant women were likely to abort, while those less severely intoxicated were more likely to deliver stillborn infants. Legge (1901), in summarizing the reports of 11 English factory inspectors, found that of 212 pregnancies in 77 female lead workers, only 61 viable children were produced. Fifteen workers never became pregnant; 21 stillbirths and 90 miscarriages occurred. Of 101 children born, 40 died in the first year. Legge also noted that when lead was fed to pregnant animals, they typically aborted. He concluded that maternal exposure to lead resulted in a direct action of the element on the fetus.

Four years later, Hall and Cantab (1905) discussed the increasing use of lead in nostrums sold as abortifacients in Britain. Nine previous reports of the use of diachylon ("lead plaster") in attempts to cause miscarriage were cited, along with 30 further cases of known or apparent use of lead in attempts to terminate real or suspected pregnancy. Of 22 cases described in detail, 12 resulted in miscarriage and all 12 exhibited marked signs of plumbism, including a blue gum line. In eight of these cases, the women were known to have attempted to induce abortion. Hall and Cantab's report was soon followed by those of Cadman (1905) and Eales (1905), who described three more women who miscarried following consumption of lead-containing pills.

Oliver (1911) then published statistics on the effect of lead on pregnancy in Britain (Table 12-13). These figures showed that the miscarriage rate was elevated among women employed in industries in which they were exposed to lead. Lead compounds were said by Taussig (1936) to be known for their embryotoxic properties and their use to induce abortion. In a more recent study by Lane (1949), women exposed to air lead levels of 750 µg/m^3 were examined for effects on reproduction. Longitudinal data on 15 pregnancies indicated an increase in the number of stillbirths and abortions. No data were given on urinary lead in these women, but men in this sample had urinary levels of 75-100 µg/liter.

TABLE 12-13. STATISTICS ON THE EFFECT OF LEAD ON PREGNANCY

Sample	Number of abortions and stillbirths per 1000 females	Number of neonatal deaths (first year) per 1000 females
Housewives	43.2	150
Female workers (mill work)	47.6	214
Females exposed to lead premaritally	86.0	157
Females exposed to lead after marriage	133.5	271

Source: Oliver (1911).

The above studies clearly indicate an adverse effect of lead at high levels on human reproductive functions, particularly miscarriages and stillbirths, when women are exposed to lead during pregnancy. Although the mechanisms underlying these effects are unknown at this time, many factors could contribute to such results. These factors range from indirect effects of lead on maternal nutrition or hormonal state before or during pregnancy to more direct gametotoxic, embryotoxic, fetotoxic, or teratogenic effects that could affect parental fertility or offspring viability during gestation. Pregnancy is a stress that may place a woman at higher risk for lead toxicity, because both iron deficiency and calcium deficiency increase susceptibility to lead, and women have an increased risk of both deficiencies during pregnancy and postparturition (Rom, 1976).

Early studies from the turn of the century generally suffer from methodological inadequacies. They must be mentioned, however, because they provide evidence that effects of lead on reproduction occurred at times when women were exposed to high levels of lead. Nevertheless, evidence for adverse reproductive outcomes in women with obvious lead poisoning is of little help in defining the effects of lead at much lower exposure levels. Efforts have been made to define more precisely the points at which lead may affect reproductive functions in both the human female and male, as well as in animals, as reviewed below.

12.6.1.2 Effects of Lead Exposure on Reproduction

12.6.1.2.1 Effects associated with exposure of women to lead. Since the time of the above reports, women have been largely, though not entirely (Khera et al., 1980), excluded from occupational exposure to lead; also, lead is no longer used to induce abortion. Thus, little new information is available on reproductive effects of chronic exposure of women to lead. Various reports (Pearl and Boxt, 1980; Qazi et al., 1980; Timpo et al., 1979; Singh et al., 1978; Angle and McIntire, 1964) suggest that relatively high prenatal lead exposures do not invariably result in abortion or in major problems readily detectable in the first few years of life.

These findings are based on only a few case histories, however, and are obviously not an adequate sample. The data are confounded by numerous variables, and longer follow-ups are needed.

In a sample population exposed to lead as well as other toxic agents from the Rönnskär smelter in Sweden, Nordström et al. (1978b) found an increased frequency of spontaneous abortions among women living closest to the smelter. In addition to the exposure to multiple environmental toxins, however, the study was confounded by failure to match exposed and control populations for socioeconomic status, which could also bear upon the women's health. A further study by the same authors (Nordström et al., 1979a) determined that female workers at the Rönnskär smelter had an increased frequency of spontaneous miscarriage when the mother was employed by the smelter during pregnancy or had been so employed prior to pregnancy and still lived near the smelter. Also, women who worked in more highly polluted areas of the smelter were more likely to have aborted than were other employees. This report, however, suffers from the same deficiencies as the earlier study.

With regard to potential effects of lead on ovarian function in human females, Panova (1972) reported a study of 140 women working in a printing plant for less than one year (1-12 months) where ambient air levels were under 7 µg Pb/m^3. Using a classification of various age groups (20-25, 26-35, and 36-40 yr) and type of ovarian cycle (normal, anovular, and disturbed lutein phase), Panova claimed that statistically significant differences existed between the lead-exposed and control groups in the age range 20-25 years. Panova's report, however, does not show the age distribution, the level of significance, or data on the specificity of her method for classification. Zielhuis and Wibowo (1976), in a critical review of the above study, concluded that the study design and presentation of data were such that it is difficult to evaluate the author's conclusions. It should also be noted that no consideration was given to the dust levels of lead, an important factor in print shops.

No other information is available for assessing the effects of lead on human ovarian function or other factors affecting female fertility. Studies offering firm data on maternal variables, e.g., hormonal state, that are known to affect the ability of the pregnant woman to carry the fetus full term are also lacking.

12.6.1.2.2 Effects associated with exposure of men to lead. Lead-induced effects on male reproductive functions have been reported in several instances. Among the earliest of these was the review of Stöfen (1974), who described data from the work of Neskov in the USSR involving 66 workers exposed chiefly to lead-containing gasoline (organic lead). In 58 men there was a decrease or disappearance of erection, in 41 there was early ejaculation, and in 44 there was a diminished number of spermatocytes. These results were confounded, however, by the presence of the other constituents of gasoline.

Lancranjan et al. (1975) reported lead-related interference with male reproductive functions. A group of 150 workmen who had long-term exposure to lead in varying degrees was studied. Clinical and toxicological criteria were used to categorize the men into four groups: lead-poisoned workmen (mean blood lead level = 74.5 µg/dl) and those showing moderate (52.8 µg/dl), slight (41 µg/dl), or "physiologic" (23 µg/dl) exposure to lead. Moderately increased lead absorption (52.8 µg/dl) was said to result in gonadal impairment. The effects on the testes were believed to be direct, in that tests for impaired hypothalamopituitary influence were negative. Also, semen analysis revealed asthenospermia and hypospermia in all groups except those with "physiologic" absorption levels, and increased teratospermia was seen in the two highest lead exposure groups.

An apparently exposure-related increase in erectile dysfunction was also found by Lancranjan et al. (1975). Problems with ejaculation and libido were said to be more common in the lead-exposed groups, but the incidence of such problems did not seem to be dose-dependent. The frequency of these problems in a control group was invariably lower than in the lead-exposed groups, however, so the lack of a clear-cut dose-response relationship may have merely been due to inappropriate assignment of individuals to the high, moderate, and low-exposure groups.

The Lancranjan et al. (1975) study has been criticized by Zielhuis and Wibowo (1976), who stated that the distributions of blood lead levels appeared to be skewed and that exposure groups overlapped in terms of lead intake. Thus, the means for each putative exposure group may not have been representative of the individuals within a group. It is difficult to discern, however, if the men were improperly assigned to exposure level groups, as blood lead levels may have varied considerably on a short-term basis. Zielhuis and Wibowo also stated that the measured urinary ALA levels were unrealistically high for individuals with the stated blood lead levels. This suggests that if the ALA values were correct, the blood lead levels may have been underestimated. Other deficiencies of the study include the failure to use matched controls and the exclusion of different proportions of individuals per exposure group for the semen analyses.

Plechaty et al. (1977) measured lead concentrations in the semen of 21 healthy men. Semen lead levels were generally less than blood lead levels, and no correlation was found between lead content of the semen and sperm counts or blood lead levels in this small sample.

Hypothalamic-pituitary-testicular relationships were investigated by Braunstein et al. (1978) in men occupationally exposed at a lead smelter. Six subjects had 2-11 years of exposure to lead and exhibited marked symptoms of lead toxicity. All had received one or more courses of EDTA chelation therapy. This group was referred to as "lead-poisoned" (LP). Four men from the same smelter had no signs of lead toxicity, but had been exposed for 1-23 years and were designated "lead-exposed" (LE). The control (C) group consisted of nine volunteers whose socioeconomic status was similar to the lead workers. Mean (± standard error) blood lead levels for the LP, LE, and C groups were 38.7 (± 3), 29.0 (± 5), and 16.1 (± 1.7) μg/dl, respectively, at the time of the study. Previously, however, the LP and LE groups had exhibited values as high as 88.2 (± 4) and 80 (± 0) μg/dl, respectively. All three groups were chelated and 24-hour urinary lead excretion values were 999 (± 141), 332 (± 17), and 225 (± 31) μg for the LP, LE, and C groups, respectively. Frequency of intercourse was significantly less in both lead-exposed groups than in controls. Sperm concentrations in semen of the LP and LE men ranged from normal to severely oligospermic, and one from the LP group was unable to ejaculate. Testicular biopsies were performed on "the two most severely lead-poisoned men," one with aspermia and one with testicular pain. Both men showed increased peritubular fibrosis, decreased spermatogenesis, and Sertoli cell vacuolization. The two lead groups exhibited reduced basal serum testosterone levels, but displayed a normal increase in serum testosterone following stimulation with human chorionic gonadotrophin. A similar rise in serum follicle-stimulating hormone was seen following treatment with clomiphene citrate or gonadotrophin-releasing hormone, although the LP men exhibited a lower-than-expected increase in luteinizing hormone (LH). The LE men also appeared to have a reduced LH response, but the decrease was not significant.

The results of the Braunstein et al. (1978) study suggest that lead exposure at high levels may result in a defect in regulation of LH secretion at the hypothalamic-pituitary level. They also indicate a likely direct effect on the testes, resulting in oligospermia and peritubular fibrosis. However, the number of subjects in the study was quite small, and there is also a possibility that the observed effects were precipitated by the EDTA chelation therapy.

More recently, Wildt et al. (1983) compared two groups of men exposed to lead in a Swedish battery factory. The high-lead men had had blood lead levels ≥50 μg/dl at least once prior to the study, while the "controls" seldom exceeded 30 μg/dl. There were two test periods, in the fall and the following spring. For the first test, 14 men in the high-lead group and 23 in the control group were examined; 16 were in each group for the second test. Fourteen and 15 of these men from the high-lead and control groups, respectively, took part in both tests. Blood lead values were obtained periodically over a six-month period. For the two high-lead groups, blood lead values were 46.1 and 44.6 μg/dl, respectively (range: 25-75); corresponding values for the controls were 31.1 and 21.5 μg/dl (range: 8-39). The high-lead men tended to exhibit decreased function of the prostate and/or seminal vesicles, as measured by seminal plasma constituents (fructose, acid phosphatase, magnesium, and zinc); however, a significant difference was seen only in the case of zinc. More men in the high-lead than in the control group had low semen volumes, but the number of subjects did not allow a reliable statistical analysis. The heads of sperm of high-lead individuals were more likely to swell when exposed to a detergent solution, sodium dodecyl sulfate (SDS), which constituted a test of functional maturity, but the results were still in the normal range. Conversely, the leakage of lactate dehydrogenase isoenzyme X (LDH-X) was greater in control semen samples.

The values for live and motile sperm were lower in the control group. The data were skewed, however, by the presence of some of the same men with low values in the control groups for both sampling times. Another confounding factor was the fact that the high-lead and control groups differed in a significant way: ten of the control men had current or past urogenital tract infections versus none in the high-lead group, possibly explaining the incidence of control samples with lowered sperm motility and viability. The observed decrease in SDS resistance in the sperm of high-lead-group men may have been related to their apparent abnormal prostatic function or to an effect of lead on sperm maturation. In evaluating the above results of Wildt et al. (1983) it must also be noted that even the "controls" had elevated blood lead levels.

When Smith et al. (1983) compared blood lead levels in a sample of 80 infertile and 38 fertile men, no differences were seen between the two groups. Also, in a sample of 15 normal and 16 vasectomized men, Butrimovitz et al. (1983) found no relationship between seminal lead and sperm count or motility. Lead levels were relatively low (3.8 and 4.1 μg/dl in the intact and vasectomized males, respectively), however, and such a result is not unexpected. At much higher levels (66-139 μg/dl), five of seven lead-poisoned men examined by Cullen et al. (1984) showed abnormal spermatogenesis, particularly oligospermia and azoospermia. Chelation therapy produced only partial improvement in these patients.

12.6.1.3 Placental Transfer of Lead. The transfer of lead across the human placenta and the consequent potential threat to the conceptus have been recognized for more than a century (Paul, 1860). Documentation of placental transfer of lead to the fetus and data on resulting fetal blood lead levels suggest a potential, but as yet not clearly defined, threat of subtle embryotoxicity or other deleterious health effects.

The placental transfer of lead has been established, in part, by various studies that have disclosed measurable quantities of lead in human fetuses or newborns, as well as offspring of experimental animals. The relevant data on prenatal lead absorption have been reviewed in Chapter 10, Section 10.2.4 of this document, and thus work dealing only with lead levels will not be discussed further here.

12.6.1.4 Effects of Lead on the Developing Human

12.6.1.4.1 Effects of lead exposure on fetal metabolism. Prenatal exposure of the conceptus to lead, even in the absence of overt teratogenicity, may be associated with biochemical effects. This is suggested by studies relating fetal and cord-blood levels to changes in enzymes and precursors related to fetal heme synthesis. Haas et al. (1972) examined 294 mother-infant pairs for blood lead and urinary ALA levels. The maternal blood lead mean was 16.89 µg/dl, while the fetal blood lead mean was 14.98 µg/dl, with a correlation coefficient of 0.54 ($p < 0.001$). In the infants, blood lead levels and urinary ALA were positively correlated ($r = 0.19$, $p < 0.01$), although the data were based on spot urines (which tends to limit their value). The full biological significance of the elevated ALA levels is not clear, but the positive correlation between lead in blood and urinary ALA for the group as a whole indicates increased potential for impairment of heme synthesis at relatively low blood lead levels in the fetus or newborn infant.

Subsequently, Kuhnert et al. (1977) measured ALA-D activity and levels of erythrocyte lead in pregnant urban women and their newborn offspring. Cord erythrocyte lead levels ranged from 16 to 67 µg/dl of cells, with a mean of 32.9. Lead levels were inversely correlated with ALA-D activity ($r = -0.58$, $p < 0.01$), suggesting that typical urban lead exposures could affect fetal enzyme activity. Note, however, that ALA-D activity is related to blood cell age and is highest in the younger cells. Thus, results obtained with cord blood, with its high percentage of immature cells, are not directly comparable to those obtained with adult blood. In a later study, Lauwerys et al. (1978) found no lead-related increase in erythrocyte porphyrin levels in 500 mothers or their offspring. They did, however, report negative correlations between ALA-D activity and blood lead levels in both mothers and their newborns. Maternal blood lead levels averaged 10.2 µg/dl, with a range of 3.1-31 µg/dl; corresponding values for the newborns were 8.4 µg/dl and 2.7-27.3 µg/dl. Such results indicate that ALA-D activity may be a more sensitive indicator of fetal lead toxicity than erythrocyte porphyrin or urinary ALA levels.

12.6.1.4.2 Other toxic effects of intrauterine lead exposure. Fahim et al. (1976), in a study on maternal and cord-blood lead levels, determined blood lead values in women having preterm delivery and premature membrane rupture. Such women residing in a "lead belt" (mining and smelting area) had significantly higher blood lead levels than women from the same area delivering at full term. Fahim et al. (1976) also noted that among 249 pregnant women in a control group outside the lead belt area, the percentages of women having preterm deliveries and premature rupture were 3.0 and 0.4, respectively, whereas corresponding values for the lead area ($n = 253$) were 13.04 and 16.99, respectively. A confusing aspect of this study, however, is the similarity of blood lead levels in women from the presumptive low-lead and lead belt areas. In fact, no evidence was presented that women in the lead belt group had actually received a greater degree of lead exposure during pregnancy than did control individuals. Also, questions exist regarding analytical aspects of this study. Specifically, other workers (e.g., see summary table in Clark, 1977) have typically found blood lead levels in mothers and their newborn offspring to be much more similar than those of Fahim et al. (1976).

In another study, Clark (1977) detected no effects of prenatal lead exposure in newborns with regard to birth weight, hemoglobin, or hematocrit. He compared children born of 122 mothers living near a Zambian lead mine with 31 controls from another area. Maternal and infant blood lead levels for the mine area were 41.2 (± 14.4) and 37.9 (± 15.3) μg/dl, respectively. Corresponding values for control mothers and offspring were 14.7 (± 7.5) and 11.8 (± 5.6) μg/dl.

Nordström et al. (1979b) examined birth weight records for offspring of female employees of a Swedish smelter and found decreased birth weights related to the following: (1) employment of the mothers at the smelter during pregnancy; (2) distance that the mothers lived from the smelter; and (3) proximity of the mother's job to the actual smelting process. Similar results were also seen for children born to mothers merely living near the smelter (Nordström et al., 1978a). Nordström et al. (1979b) also investigated birth defects in offspring of the female smelter workers and in populations living at various distances from the smelter. They concluded that the frequencies of both single and multiple malformations were increased when the mother worked at the smelter during pregnancy.

The number of smelter workers with malformed offspring was relatively small (39 of 1291). The incidence of children with birth defects whose mothers worked while pregnant was 5.8 percent (17 of 291). Five of the six offspring with multiple malformations were in this group, suggesting that the observed effect may have been a real one. Nevertheless, a crucial factor in evaluating all of these results is the exposure of workers and the nearby population to a number of toxic substances, including not only lead, but arsenic, mercury, cadmium, and sulfur dioxide as well.

Alexander and Delves (1981) found that the mean blood lead concentrations of pregnant and non-pregnant women living in an urban area of England were approximately 4 μg/dl higher than those for similar groups living in a rural area. The mean concentrations for the urban and rural pregnant women were 15.9 and 11.9 μg/dl, respectively ($p < 0.001$), but there were no demonstrable effects of the higher maternal blood lead levels on any aspect of perinatal health. The rate for congenital abnormality was higher in the rural area, suggesting that whatever the cause, it was unlikely to be related to maternal levels of lead.

Khera et al. (1980) measured placental and stillbirth tissue lead in occupationally exposed women in the United Kingdom. Regardless of the incidence of stillbirths, placental lead concentrations were found to increase with duration of occupational exposure, from 0.29 μg/g at <1 year exposure to 0.48 μg/g at >6 years exposure for a group of 26 women aged 20-29 years. Placental lead concentrations also increased with age of the mother, independently of time of occupational exposure, and ranged from 0.30 (± 0.16) μg/g for those <20 years old to 0.51 (±0.44) μg/g for those ≥30 years old. Average placental lead concentrations for 20 occupationally exposed women whose babies were stillborn were higher [0.45 (± 0.32) μg/g] than the average level [0.29 (± 0.09) μg/g] for placentas from eight mothers who had not been occupationally exposed for at least two years. The authors noted, however, that it was not possible to say whether occupational exposure caused any of the stillbirths or whether the high lead levels were merely consequential to the fetal death. Interpretation of this study is also somewhat complicated by the fact that the average placental lead concentration was about one-third that reported earlier by this group (Wibberley et al., 1977). These differences were attributed to methodological changes and to changes in concentration during storage of placentas at -20°C (Khera et al., 1980).

A study by Roels et al. (1978b) reported placental lead values of 0.08 (± 0.05) μg/g (range = 0.01-0.40 μg/g) from a variety of locations in Belgium, but these data indicated no correlation between lead concentration and birth weight. In contrast, placental lead has been reported to be associated with decreased activity of a placental enzyme, steroid sulfatase (Karp and Robertson, 1977). A similar association was found for mercury, suggesting that either metal or both together could have affected the enzyme activity or that the authors had merely uncovered a spurious correlation. There is also some evidence that lead levels in bone samples from stillborn children are higher than would otherwise be expected (Khera et al., 1980; Bryce-Smith et al., 1977), but the data are inconclusive. (See the Addendum to this document for a discussion of more recent evidence concerning possible effects of intrauterine lead exposure on prenatal and postnatal development in children.)

12.6.1.4.3 Paternally mediated effects of lead. There is increasing evidence that exposure of male laboratory animals to toxic agents can result in adverse effects on their offspring, including decreased litter size, birth weight, and survival (see Section 12.6.2.2.1). Mutagenic effects are the most likely cause of such results, but other mechanisms have been proposed (Soyka and Joffe, 1980). In the following cases, exposure of human males to lead has been implicated as the cause of adverse effects on the conceptus.

According to Koinuma (1926) in a brief report, 24.7 percent of workmen exposed to lead in a storage battery plant had childless marriages, while the value for men not so exposed was 14.8 percent. Rates for miscarriages or stillbirths in wives of lead-exposed men and controls were 8.2 and 2.8 percent, respectively, while corresponding figures for neonatal deaths were 24.2 and 19.2 percent. The comparisons were based on 170 lead-exposed and 128 control men. These differences in fertility and prenatal mortality, while not dramatic, are suggestive of a male-mediated lead effect; however, the reliability of the methodology used in this study cannot be determined, due to the brevity of the report.

In a study of the pregnancies of 104 Japanese women before and after their husbands began lead-smelter work, miscarriages increased to 8.3 percent of pregnancies from a pre-exposure rate of 4.7 percent (Nogaki, 1957). The miscarriage rate for 75 women whose husbands were not occupationally exposed to lead was 5.8 percent. In addition, exposure to lead was related to a significant increase in the ratio of male to female offspring at birth. Lead content of paternal blood ranged from 11 to 51.7 μg/dl [mean = 25.4 (± 1.26) μg/dl], but was not correlated with reproductive outcome, except in the case of the male-to-female offspring ratio. The reported blood lead levels appear low, however, in view of the occupational exposure of these men, and were similar to those given for controls [mean = 22.8 (± 1.63) μg/dl]. Also, maternal age and parity appear not to have been well controlled for in the analysis of the data on reproductive outcome. Another report (Van Assen, 1958) on fatal birth defects in children conceived during a period when their father was lead-poisoned (but neither before nor after) also suggests but does not clearly demonstrate paternally-mediated effects of lead.

In the study by Nordström et al. (1979b), women employed at the Rönnskär smelter in Sweden were found to have higher miscarriage rates if their husbands were also employed at the smelter. This was true only of their third or later pregnancies, however, suggesting that the effect was related to long-term exposure of the male gametogenic stem cells. Whether this was a lead effect or that of other toxins from the smelter is not clear.

12.6.1.5 Summary of the Human Data. The literature on the effects of lead on human reproduction and development leaves little doubt that lead can, at high exposure levels, exert significant adverse health effects on reproductive functions. Most studies, however, only examined the effects of prolonged moderate to high exposure to lead, such as that encountered in industrial situations, and many reports do not provide definite information on exposure levels or blood lead levels at which specific effects were observed. Also, the human data were largely derived from studies involving relatively small numbers of individuals and therefore do not allow for discriminating statistical analysis. These reports are often additionally confounded by the failure to include appropriate controls and, in some cases, by the presence of additional toxic agents or disease states. These and other factors obviously make interpretation of the data difficult. Based on the Lancranjan et al. (1975) and Wildt et al. (1983) studies, it appears possible that effects on sperm or the testis may result from chronic exposure at blood lead values of 40-50 μg/dl, but additional data are greatly needed. Exposure data related to reproductive functions in the female are so lacking that even a rough estimate is impossible. Data on maternal exposure levels at which effects may be seen in human fetuses or infants are also quite meager or equivocal. However, the results of Haas et al. (1972), Kuhnert et al. (1977), and Lauwerys et al. (1978) suggest possible perinatal effects on heme metabolism at maternal blood levels considerably below 30 μg/dl. [More recent studies dealing with the effects of relatively low-level lead exposure on development both prenatally (e.g., congenital anomalies, birth weight, gestational age) and postnatally (e.g., infant mental development, child stature) are reviewed in an Addendum to this document.]

12.6.2 Animal Studies

12.6.2.1 Effects of Lead on Reproduction

12.6.2.1.1 Effects of lead on male reproductive functions. Among the first investigators to report infertility in male animals due to lead exposure were Puhac et al. (1963), who exposed rats to lead via their diet. Ability to sire offspring returned, however, 45 days after cessation of treatment. More recently, Varma et al. (1974) gave a solution of lead subacetate in drinking water to male Swiss mice for four weeks (mean total intake of lead = 1.65 g). The fertility of treated males was reduced by 50 percent. Varma and coworkers calculated the mutagenicity index (number of early fetal deaths/total implants) to be 10.4 for lead-treated mice versus 2.98 for controls ($p < 0.05$). The major differences in fecundity appeared to have been due to differing pregnancy rates, however, rather than prenatal mortality. Impairment of male fertility by lead rather than lead-induced mutagenicity was thus likely to have been the primary toxic effect observed. Additionally, it has been suggested by Léonard et al. (1973), that effects seen following administration of lead acetate in water may be due to resulting acidity, rather than to lead. Also, Eyden et al. (1978) found no decrease in fertility of male mice fed 0.1 percent lead acetate in the diet for 64 weeks.

Several animal studies have found lead-associated damage to the testes or prostate, generally at relatively high doses. Golubovich et al. (1968) found a decrease in normal spermatogonia in the testes of rats gavaged for 20 days with lead (2 mg/kg per day). Desquamation of the germinal epithelium of the seminiferous tubules was also increased, as were degenerating spermatogonia. Hilderbrand et al. (1973) also noted testicular damage in male rats given oral lead (100 µg/day for 30 days). Egorova (cited in Stöfen, 1974) injected lead at a dose of 2 µg/kg six times over a ten-day period and reported testicular damage.

Ivanova-Chemishanska et al. (1980) investigated the effect of lead on male rats administered 0.0001 or 0.01 percent solutions of lead acetate over a four-month period. The authors reported that changes in enzymatic activity and in levels of disulfide and ATP were observed in testicular homogenates. No histopathological changes in testicular tissue were found, but the fertility index for treated males was decreased. Offspring of those males exhibited postpartum "failure to thrive" and stunted growth. Such data suggest biological effects due to chronic lead exposure of the male, but the study is difficult to evaluate due to limited information on the experimental methods, particularly the dose levels actually received.

In a more recent study of lead's effects on the male reproductive tract, Chowdhury et al. (1984) found testicular atropy along with cellular degeneration in rats exposed to lead acetate in water at 1 g/l for 60 days. Blood lead level at that exposure averaged 142.6 µg/dl. At a lower exposure level yielding a blood lead concentration of 71.7 µg/dl, seminiferous tubular diameter was singificantly reduced, as was spermatid count. No significant changes were seen at a blood lead level of 54.0 µg/dl.

Non-rodent species have also been investigated. No histopathological changes were seen during an examination of the testes of rabbits (Willems et al., 1982). Five males per group were dosed subcutaneously with up to 0.5 mg/kg lead acetate three times weekly for 14 weeks. Blood lead levels at termination of treatment were 6.6 and 61.5 µg/dl for control and high-dose rabbits, respectively.

Lead-related effects on spermatozoa have also been reported. For example, Stowe et al. (1973) described the results of a low-calcium and phosphate diet containing 100 ppm lead (as acetate) fed to dogs from 6 to 18 weeks of age. This dose resulted in a number of signs of toxicity, including spermatogonia with hydropic degeneration. In a study by Maisin et al. (1975), male mice received up to 1 percent lead in the diet, and the percentage of abnormal spermatozoa increased with increasing lead exposure. Eyden et al. (1978) also fed 1 percent lead acetate in the diet to male mice. By the eighth week, abnormal sperm had increased; however, the affected mice showed weight loss and other signs of general toxicity. Thus, the effect on spermatogenesis was not indicative of differential sensitivity of the gonad to lead.

Krasovskii et al. (1979) observed declines in motility, duration of motility, and osmotic stability of sperm from rats given 0.05 mg/kg lead orally for 20-30 days. Damage to gonadal blood vessels and to Leydig cells was also seen. Rats treated for 6-12 months exhibited abnormal sperm morphology and decreased spermatogenesis. In the report of Willems et al. (1982) described above, however, no effects on sperm count or morphology were seen in rabbits.

Lead acetate effects on sperm morphology were also tested in mice given about one sixteenth to one half an LD_{50} dose by i.p. injection on five consecutive days (Bruce and Heddle, 1979; Wyrobek and Bruce, 1978; Heddle and Bruce, 1977). The two lowest doses (apparently 100 and 250 mg/kg) resulted in only a modest increase in morphologically abnormal sperm 35 days after treatment, but the 500 or 900 mg/kg doses resulted in up to 21 percent abnormal sperm.

That lead could directly affect developing sperm or their cellular precursors is made more plausible by the data of Timm and Schulz (1966), who found lead in the seminiferous tubules of rats and in their sperm. The mechanisms for lead's effects on the male gonad or gamete are unknown, although Golubovich et al. (1968) found altered RNA levels in the testes of lead-exposed rats. They suggested that testicular damage was related to diminished ribosomal activity and inhibition of protein synthesis. As noted above, Ivanova-Chemishanska et al. (1980) observed biochemical changes in testes of lead-treated mice. Nevertheless, such observations are only initial attempts to determine a mechanism for the observed effects of lead. A more likely mechanism for such effects on the testes may be found in the work of Donovan et al. (1980), who found that lead inhibited androgen binding by the cytosolic receptors of mouse prostate. This could provide a mechanism for the observation of Khare et al. (1978), who found that injection of lead acetate into the rat prostate resulted in decreased prostatic weight; no such changes were seen in other accessory sex glands or in the testes.

Effects on hormonal production or on hormone receptors could also explain the results of Maker et al. (1975), who observed a delay in testicular development and an increase in age of first mating in male mice of two strains whose dams were given 0.08 percent lead (C57B1/6J) or 0.5 percent lead (Swiss-Webster albino) during pregnancy and lactation. The weanling males were fed these same doses in their diets through 60 days of age.

In attempts to further examine the possible mechanisms of effects on the male, Wiebe et al. (1982) treated rats with lead acetate injected s.c. from gestation day 9 every 3-4 days throughout pregnancy and for the first 2-3 weeks of lactation. Testes from the two- to three-week-old male offspring of treated mothers had normal weights and seminiferous tubule diameters, but yielded testicular homogenates with decreased ability to convert progesterone to a variety of metabolites. Such results, in addition to direct enzyme assays, showed decreased activities of 3α-, 3β-, and 20α-hydroxysteroid oxidoreductases and of the 5α-reductase and C_{17}-$_{20}$-lyase enzymes. Receptor binding of FSH was also significantly reduced. More recently, Wiebe et al. (1983) compared Sertoli cells isolated from prepubertal rats and cultured in the presence of either the acetate salts of lead or sodium (2.64×10^{-4} M). After 24 hours, lead exposure was associated with a 10-20 percent decrease in FSH binding and in the production of cyclic AMP; at 96 hours, the decrease was 75 percent. Sixteen-day-old rats were more sensitive than those 20 days of age and exhibited a 97 percent decrease in FSH-induced cyclic AMP by 144 hours of lead exposure. The ability of Sertoli cells to metabolize progesterone and their steroidogenic response to FSH was also inhibited by 48-hour lead exposure. Activity of cellular 3β-hydroxysteroid dehydrogenase was decreased after lead exposure in Sertoli cells and in vitro in the presence of $PbCl_2$ in the assay buffer. These results support the concept that lead may directly affect testicular enzymes or may act indirectly by a reduction in testicular binding of FSH and production of cyclic AMP.

Another potential mechanism underlying lead's effects on sperm involves its affinity for sulfhydryl groups. Mammalian sperm possess high concentrations of sulfhydryls believed to be involved in the maintenance of motility and maturation via regulation of stability in sperm heads and tails (Bedford and Calvin, 1974; Calvin and Bedford, 1971). It has also been found that blockage of membrane thiols inhibits sperm maturation (Reyes et al., 1976).

12.6.2.1.2 Effects associated with exposure of females to lead. Numerous studies have focused on lead exposure effects in females. For example, effects of lead on reproductive functions of female rats were studied by Hilderbrand et al. (1973), using animals given lead acetate orally at doses of 5 or 100 μg for 30 days. Control rats of both sexes had the same blood lead levels. Blood lead levels of treated females were higher than those of similarly treated males: 30 versus 19 μg/dl at the low dose, and 53 versus 30 μg/dl at the high dose. The females exhibited irregular estrous cycles at both doses. When blood lead levels reached 50 μg/dl, they developed ovarian follicular cysts, with reductions in numbers of corporalutea.

In a subsequent study (Der et al., 1974), lead acetate (100 µg lead per day) was injected subcutaneously for 40 days in weanling female rats. Treated rats received a low-protein (4 percent) or adequate-protein (20 percent) diet; controls were given the same diets without lead. Females on the low-protein, high-lead diet did not display vaginal opening during the treatment period and their ovaries decreased in weight. No estrous cycles were observed in animals from either low-protein group; those of the adequate diet controls were normal, while those of the rats given adequate protein plus lead were irregular in length. Endometrial proliferation was also inhibited by lead treatment. Blood lead levels were 23 µg/dl in the two control groups, while values for the adequate- and low-protein lead-treated groups were 61 and 1086 µg/dl, respectively. The reports of Hilderbrand et al. (1973) and Der et al. (1974) suggest that lead chronically administered in high doses can interfere with sexual development in rats and the body burden of lead is greatly increased by protein deprivation.

Maker et al. (1975) noted a delay in age at first conception in female mice of two strains exposed to 0.08 percent (C57B1/6J) or 0.5 percent lead (Swiss-Webster) indirectly via the maternal diet (while *in utero* and nursing) and directly up to 60 days of age. These females were retarded in growth and tended to conceive only after reaching weights approximating those at which untreated mice normally first conceive. Litters from females that had themselves been developmentally exposed to at least 0.5 percent lead had lower survival rates and retarded development. More recently, Grant et al. (1980) reported delayed vaginal opening in rats whose mothers were given 25, 50, or 250 ppm lead (as lead acetate) in their drinking water during gestation and lactation followed by equivalent exposure of the offspring after weaning. The vaginal opening delays in the 25-ppm females occurred in the absence of any growth retardation or other developmental delays and were associated with median blood lead levels of 18-29 µg/dl.

Although most animal studies have used rodents, Vermande-Van Eck and Meigs (1960) administered lead chloride intravenously to female rhesus monkeys. The monkeys were given 10 mg/week for four weeks and 20 mg/week for the next seven months. Lead treatment resulted in cessation of menstruation, loss of color of the "sex skin" (presumably due to decreased estrogen production), and pathological changes in the ovaries. One to five months after lead treatment ceased, menstrual periods resumed, the sex skin returned to a normal color, and the ovaries regained their normal appearance. Thus, there was an apparent reversal of these effects on female reproductive functions, although there were no confirmatory tests of fertility.

The above studies indicate that pre- and/or postnatal exposure of female animals to lead can affect pubertal progression and hypothalamic-pituitary-ovarian-uterine functions. The observations of delayed vaginal opening may reflect delayed ovarian estrogen secretion due to toxicity to the ovary, hypothalamus, or pituitary. One study has demonstrated decreased levels of circulating follicle-stimulating hormone (Petrusz et al., 1979), and others discussed previously have shown lead-induced ovarian atrophy (Stowe and Goyer, 1971; Vermande-Van Eck and Meigs, 1960), again suggesting toxicity involving the hypothalamic-pituitary-ovarian-endometrial axis.

12.6.2.2 Effects of Lead on the Offspring. This section discusses developmental studies of animals whose parents (one or both) were exposed to lead. Possible male-mediated effects as well as effects of exposure during gestation are reviewed. Results obtained for offspring of females given lead following implantation or throughout pregnancy are summarized in Tables 12-14 and 12-15.

12.6.2.2.1 Male-mediated effects. A few studies have focused on male-mediated lead effects on the offspring and have suggested that paternally transmitted effects of lead may cause reductions in litter size, offspring weight, and survival rate.

Cole and Bachhuber (1915), using rabbits, were the first to report paternal effects of lead intoxication. In their study, the litters of dams sired by lead-intoxicated male rabbits were smaller than those sired by controls. Weller (1915) similarly demonstrated reduced birth weights and survival among offspring of lead-exposed male guinea pigs.

Offspring of lead-treated males from the Ivanova-Chemishanska et al. (1980) study described above were affected in a variety of ways, e.g., they exhibited "failure to thrive" and lower weights than did control progeny at one and three weeks postpartum. These results are difficult to interpret, however, without more specific information on the experimental methods and dosing procedures.

12.6.2.2.2 Results of lead exposure of both parents. Only a few studies have assessed the effects of lead exposure of both parents on reproduction. Schroeder and Mitchener (1971) found a reduction in the number of offspring of rats and mice given drinking water containing 25 ppm lead. According to the data of Schroeder et al. (1970), however, animals in the 1971 study may have been chromium-deficient, and the Schroeder and Mitchener (1971) results are in marked contrast to those of an earlier study by Morris et al. (1938), who reported no significant reduction in weaning percentage among offspring of rats fed 512 ppm lead.

In another study, Stowe and Goyer (1971) assessed the relative paternal and maternal effects of lead as measured by effects on the progeny of lead-intoxicated rats. Female rats fed diets with or without 1 percent lead were mated with normal males. The pregnant rats were continued on their respective rations with or without lead throughout gestation and lactation. Offspring of these matings, the F_1 generation, were fed the rations of their dams and were mated in combinations as follows: control female to control male (CF-CM), control female to lead-intoxicated male (CF-PbM), lead-intoxicated female to control male (PbF-CM), and lead-

TABLE 12-14. EFFECTS OF PRENATAL EXPOSURE TO LEAD ON THE OFFSPRING OF LABORATORY AND DOMESTIC ANIMALS: STUDIES USING ORAL OR INHALATION ROUTES OF EXPOSURE

Species	Test agent	Treatment		Effect on the offspring[a]			Reference
		Dose and mode[b]	Timing[c]	Mortality	Fetotoxicity	Malformation	
Rat	Lead acetate	512 ppm in diet	all	-	-	?	Morris et al. (1938)
		10,000 ppm in diet	all	+	+	?	Stowe and Goyer (1971)
		39 mg/kg/day, po	6-16	-	-	-	Kennedy et al. (1975)
		390 mg/kg/day, po	6-8	+	+	-	
		255-478 mg/kg/day in water	all,LAC	?	+[d]	?	Murray et al. (1978)
		31.9-319 ppm in water	all	±[e]	+	-	Dilts and Ahokas (1979, 1980)
		50-250 ppm in water	all, LAC	-	+	-	Kimmel et al. (1980)
		25 ppm in water	all, LAC	-	±	-	
		0.5-5 ppm in water	all, LAC	-	-	-	
		5 or 50 ppm in water	all, LAC	+	+	-	Herman et al. (1981)
		31.9-47.8 mg/kg/day, po	all	-	-	-	Miller et al. (1982)
		63.7 mg/kg/day, po	all	-	+	-	
		150 mg/kg/day, po	6-18	+	-	-	Wardell et al. (1982)
		500 ppm in water	1-18 or 1-21	-	+	-	Hayashi (1983a)
		5 ppm in water	1-18 or 1-21	-	+	-	Hayashi (1983b)
		5-500 ppm in water	all, LAC	-	+	-	Victery et al. (1983)
	Tetraethyl lead	1.6-3.2 mg/kg/day, po	9-11 or 12-14	±	+	-	McClain and Becker (1972)
		0.064 mg/kg/day, po	6-16	-	-	-	Kennedy et al. (1975)
		0.64 mg/kg/day, po	6-16	+	+	-	
		6.4 mg/kg/day, po	6-8	+	+	-	
	Tetramethyl lead	10-28.7 mg/kg/day, po	9-11 or 12-14	±	+	-	McClain and Becker (1972)
	Trimethyl lead chloride	3.6-7.2 mg/kg/day, po	9-11 or 12-14	-	+	-	
	Lead nitrate	1 ppm in water	all	-	-[f,g]	?	Hubermont et al. (1976)
		10 ppm in water	all	-	+	?	
	Lead (aerosol)	1 or 3 mg/m³, inhaled	1-21	?	-[f]	?	Prigge and Greve (1977)
		10 mg/m³, inhaled	1-21	?	+[h]	?	

TABLE 12-14. (continued)

Species	Test agent	Treatment		Effect on the offspring[a]			Reference
		Dose and mode[b]	Timing[c]	Mortality	Fetotoxicity	Malformation	
Mouse	Lead acetate	3,185 ppm in diet	1-7	+	±	N/A	Jacquet (1977)
		780-1,593 ppm in diet	1-16,17, or 18	?	+[f,i]	?	Jacquet et al. (1977b)
		3,185 ppm in diet	1-16,17, or 18	?	+[f,i,j]	?	
		1,593-6,370 ppm in diet	1-15,16, or 17	?	+[k]	?	Gerber and Maes (1978)
		1,595-3,185 ppm in diet	7-16,17, or 18	?	+[l]	?	Gerber et al. (1978)
		39 mg/kg/day, po	5-15	-	-	-	Kennedy et al. (1975)
		390 mg/kg/day, po	5-7	+	+	-	
		0.1-1.0 g/l in water	all	-	?	?	Léonard et al. (1973)
		637-3,185 ppm in diet	1-18	+	?	?	Maisin et al. (1975)
		1,593 ppm in diet	1-16,17, or 18	+	-	-	Jacquet et al. (1975)
		3,185 ppm in diet	1-16,17, or 18	+	+	-	
		1,250 ppm in diet	all	-	+	-	
		3,185 ppm in diet	1-16,17, or 18	+	+	-	
		1,250 ppm in diet	all	-	+	-	
		2,500-5,000 ppm in diet	all	+	+	-	Jacquet (1976)
		1,250 ppm in diet	all	-	+	-	Talcott and Koller (1983)
		1,000 ppm in diet	all, LAC	-	-	-	
	Tetraethyl lead	0.06 mg/kg/day, po	6-16	-	-	-	Kennedy et al. (1975)
		0.64 mg/kg/day, po	6-16	+	+	-	
		6.4 mg/kg/day, po	6-8	+	+	-	
Sheep	Lead powder	0.5-16 mg/kg/day, in diet[l]	all	+	?	-	Sharma and Buck (1976)

[a] + = present; - = effect not seen; ± = ambiguous effect; ? = effect not examined or insufficient data.

[b] Dose as elemental lead; po = per os (gavage).

[c] Specific gestation days when exposed; LAC = also during lactation.

[d] Decreased numbers of dendritic spines and malformed spines at day 30 postpartum.

[e] Litter size values for high-dose group suggestive of an effect.

[f] ALA-D activity was decreased.

[g] Free tissue porphyrins increased in kidneys.

[h] Hematocrit was decreased.

[i] Fetal porphyrins were increased, except in the low-dose fetuses assayed on gestation day 18.

[j] Decreased heme and fetal weight.

[k] Incorporation of Fe into heme decreased, and growth was retarded.

[l] Decreased placental blood flow.

TABLE 12-15. EFFECTS OF PRENATAL LEAD EXPOSURE ON OFFSPRING OF LABORATORY ANIMALS: RESULTS OF STUDIES EMPLOYING ADMINISTRATION OF LEAD BY INJECTION

		Treatment		Effect on the offspring[a]			
Species	Test agent	Dose and mode[b]	Timing[c]	Mortality	Fetotoxicity	Malformation	Reference
Rat	Lead acetate	15.9 mg/kg, ip	9	+	+	+	Zegarska et al. (1974)
	Lead nitrate	31.3 mg/kg, iv	8	-	+	+	McClain and Becker (1975)
		31.3 mg/kg, iv	9 or 16	+	+	+	
		31.3 mg/kg, iv	10-14, 15,17	+[d]	+	-	
		3.13 mg/kg, iv	9 or 15	-	-	-	Hackett et al. (1978, 1979)
		15.6 mg/kg, iv	9	+	+	+	
		15.6 mg/kg, iv	15	+	?	?	
		unknown, iv	8 or 9	+	?	+	Coro Antich and Amoedo Mon (1980)
		31.3 mg/kg, iv	17	-	+	-	Minsker et al. (1982)
		15.6 mg/kg, iv	17	+	+	-	
		5 mg/kg, iv	9 or 15	-	-	-	Hackett et al. (1982a,b)
		25 mg/kg, iv	9 or 15	+	+	+,-[e]	
	Lead chloride	7.5 mg/kg,[f]	9	±	-	-	McLellan et al. (1974)
		75 mg/kg,[f]	9	+	+	-	
	Trimethyl lead chloride	20.2 mg/kg, iv	12	-	+	-	
		23.8 mg/kg, iv	9,10,13, or 15	+[g]	+	-	
Mouse	Lead acetate	9.56-22.3 mg/kg, ip	8	-	+	+	Jacquet and Gerber (1979)
		9.56 mg/kg, ip	9	+	-	+	
		22.3 mg/kg, ip	9	+	+	+	
		22.3 mg/kg, ip	10 or 12	-	-	-	
	Lead chloride	29.8 mg/kg, iv	3 or 4	+	?	?	Wide and Nilsson (1977)
		29.8 mg/kg, iv	6	+	N/A	N/A	

TABLE 12-15. (continued)

Species	Test agent	Treatment		Effect on the offspring[a]			Reference
		Dose and mode[b]	Timing[c]	Mortality	Fetotoxicity	Malformation	
Hamster	Lead acetate	31.9 mg/kg, iv	8	+	?	+	Ferm (1969)
	Lead acetate or chloride	31.9 or 37.3 mg/kg, iv	8	?	?	+	Ferm and Carpenter (1967)
	Lead nitrate	31.3 mg/kg, iv	7, 8, or 9	?	?	+	Ferm and Carpenter (1967)
		15.6-31.3 mg/kg, iv	8 or 9	+	?	+	Ferm and Ferm (1971)
		31.3 mg/kg, iv	8	+	+	+	Carpenter and Ferm (1977)
		31.3 mg/kg, iv	8	+	+[h]	+	Gale (1978)

[a] + = effect present; - = effect not seen; ± = ambiguous effect; ? = effect not examined or insufficient data.

[b] Dose as elemental lead; ip = intraperitoneally; iv = intravenously.

[c] Specific gestation days when exposed.

[d] With the exception of day 17.

[e] No fetuses survived to be examined for malformation.

[f] No dosage route specified.

[g] Only after day 10 treatment.

[h] Delayed ossification (fetal weights not given).

intoxicated female to lead-intoxicated male (PbF-PbM). The results of this study are shown in Table 12-16.

The paternal effects of lead included reductions of 15 percent in the number of pups per litter, 12 percent in mean pup birth weight, and 18 percent in pup survival rate. The maternal effects of lead included reductions of 26 percent in litter size, 19 percent in pup birth weight, and 41 percent in pup survival. The combined male and female effects of lead toxicity resulted in reductions of 35 percent in the number of pups per litter, 29 percent in pup birth weight, and 67 percent in pup survival to weaning. Stowe and Goyer classified the effects of lead upon reproduction as gametotoxic, intrauterine, and extrauterine. The gametotoxic effects of lead seemed to be irreversible and had additive male and female components. Intrauterine effects were presumed to be due to lead uptake by the conceptus, plus gametotoxic effects. The extrauterine effects were due to the passage of lead from the dam to the nursing pups, adding to the gametotoxic and intrauterine effects.

Léonard et al. (1973), however, found no effect on the reproductive performance of groups of 20 pairs of mice given lead in their drinking water over a nine-month period. Lead doses ranged from 0.1 to 1.0 g/l. A total amount of 31 g/kg was ingested at the high dose, equivalent to ingestion of 2.2 kg lead by a 70-kg man over the same time period.

More recently, rats from mothers that were exposed to lead at 5 or 50 ppm or to lead plus cadmium at concentrations of 5 ppm Pb + 0.1 ppm Cd or 50 ppm Pb + 5 ppm Cd in the drinking water during gestation and lactation were themselves continued on the same treatments (Herman et al., 1981). All individuals were treated during mating, with the mated females also being treated during gestation and given a teratological examination at day 20. Other females were allowed to litter and treatment was continued through postpartum day 21. Treatment with lead or lead plus cadmium appeared to cause preimplantation loss. In the groups allowed to litter, maternal weight gain, litter size, and offspring survival and weight were all said to be reduced in both lead groups and more severely in the Pb plus Cd groups. Eye opening was also delayed in all groups. The value of these results is not clear, however, as no statistical analysis was mentioned.

12.6.2.2.3 Lead effects on implantation and early development. Numerous studies have been performed to elucidate mechanisms by which lead causes prenatal death. They suggest two mechanisms of action for lead, one on implantation and the other (mainly at higher doses) on fetal development. The latter is discussed primarily in Section 12.6.2.2.4.5.

Maisin et al. (1975) exposed female mice to dietary lead for 18 days after mating; the number of both pregnancies and surviving embryos decreased. Similarly, exposure of female mice to lead via their diet (0.125-1.0 percent) from mating to 16-18 days afterward (Jacquet, 1976; Jacquet et al., 1975) resulted in the following: decreased incidence of pregnancy and number of corpora lutea; increased number of embryos dying after implantation at the highest dosages; decreased body weights of surviving fetuses; and fatalities among treated dams at the

TABLE 12-16. REPRODUCTIVE PERFORMANCE OF F_1 LEAD-INTOXICATED RATS (MEANS ± STANDARD ERRORS)

Parameter	Type of mating^{a}			
	CF-CM	CF-PbM	PbF-CM	PbF-PbM
Litters observed	22	24	36	16
Pups per litter	11.90 ± 0.40	10.10 ± 0.50	8.78 ± 0.30^{b}	7.75 ± 0.50^{c}
Pup birth weight, g	6.74 ± 0.15	5.92 ± 0.13^{c}	5.44 ± 0.13^{c,d}	4.80 ± 0.19^{c,d,e}
Weaned rats per litter	9.84 ± 0.50	7.04 ± 0.77^{c}	5.41 ± 0.74^{c,d}	2.72 ± 0.70^{c,d,e}
Survival rate, %	89.80 ± 3.20	73.70 ± 7.90	52.60 ± 7.20	30.00 ± 8.20^{c,d,f}
$\frac{\text{Litter birth weight}}{\text{Dam breeding weight}}$, %	28.04 ± 1.30	22.30 ± 0.90^{c}	19.35 ± 1.00^{c}	15.38 ± 1.10^{c,d,f}
$\frac{\text{Litter birth weight}}{\text{Dam whelping weight}}$, %	19.09 ± 0.80	15.97 ± 0.58^{c}	14.28 ± 0.66^{c}	11.58 ± 0.78^{c,d,f}
$\frac{\text{Gestational gain}}{\text{Pups per litter}}$, g	11.54 ± 0.60	11.20 ± 0.74	11.17 ± 0.54	12.34 ± 1.24
Nonfetal gestational gain per fetus, g	3.93 ± 0.38	4.83 ± 0.47	4.15 ± 0.42	3.96 ± 0.46

[a] CF = control female; CM = control male; PbM = lead-treated male; PbF = lead-treated female.

[b] Significantly ($p < 0.05$) less than mean for CF-CM.

[c] Significantly ($p < 0.01$) less than mean for CF-CM.

[d] Significantly ($p < 0.01$) less than mean for CF-PbM.

[e] Significantly ($p < 0.01$) less than mean for PbF-CM.

[f] Significantly ($p < 0.05$) less than mean for PbF-CM.

Source: Stowe and Goyer (1971).

high dose. Jacquet and co-workers described effects of maternal dietary lead exposure on preimplantation mouse embryos (Jacquet, 1976; Jacquet et al., 1976). They found lead in the diet to be associated with retardation of cleavage in embryos, failure of trophoblastic giant cells to differentiate, and absence of a uterine decidual reaction. Maisin et al. (1978) also found delayed cleavage in embryos of mice fed lead acetate prior to mating and up to 7 days afterwards.

Giavini et al. (1980) further confirmed the ability of lead to affect the preimplantation embryo in studies of rats transplacentally exposed to lead nitrate, and Wide and Nilsson (1977, 1979) reported that inorganic lead had similar effects on mice. Jacquet (1978) was able to force implantation in that species by use of high doses of progesterone, while Wide (1980) determined that administration of estradiol-17β and progesterone could reverse the effects of lead on implantation. Wide suggested that the lead-induced implantation blockage was mediated by a decrease in endometrial responsiveness to both sex steroids. Jacquet (1976) and Jacquet et al. (1977b) had attributed lead-induced prevention of implantation in the mouse to a lack of endogenous progesterone alone, stating that estrogen levels were unaffected. Later, however, Jacquet et al. (1977a) stated that estrogen levels also decreased, a finding not supported by Wide and Wide (1980). The latter authors did find a lead-induced increase in uterine estradiol receptors, but no change in binding affinities. Although sex steroids appear to be involved in lead's effects on implantation in rodents, the precise mechanism is not clear.

In order to examine lead's effects early in gestation, Wide and Nilsson (1977) examined embryos from untreated mice and from mothers given 1 mg lead chloride on days 3, 4, or 6 of pregnancy. Embryonic mortality was greater in lead-treated litters; in the day-6 group some abnormal embryos were observed by day 8. In a later experiment, Wide (1978) removed blastocysts from lead-treated mice. She found that they attached and grew normally during three days of in vitro culture. Other blastocysts from untreated mothers were cultured in media containing lead, and a dose-dependent decrease in the number of normally developing embryos was seen. Wide (1983) then transplanted blastocysts from mice treated with an implantation-inhibiting lead dose and found that they implanted and developed normally in foster mothers.

In a more recent study, Molls et al. (1983) exposed two-cell mouse embryos to 0.1 or 1.0 μg lead chloride per ml of culture medium. By 64 hours of incubation, both treatment levels had resulted in decreased cell proliferation. Cell death was also seen in morula stage lead-treated embryos. Exposure to x-rays one hour after the start of incubation had an additional (but not synergistic) effect.

A study employing domestic sheep was reported by Sharma and Buck (1976), who fed lead powder to pregnant ewes throughout gestation. Levels in the diet were varied from 0.5 to 16 mg/kg per day in an effort to keep blood lead levels near 40 μg/dl (actual levels ranged from

30 to 70 μg/dl). Such treatment resulted in a greatly decreased lambing percentage but no gross malformations. However, only 11 lead-treated and 9 control subjects were studied.

12.6.2.2.4 Teratogenicity and prenatal toxicity of lead in animals.

12.6.2.2.4.1 High dose effects on the conceptus. Teratogenic effects refer to physical defects (malformations) in the developing offspring. Prenatal toxicity (embryotoxicity, fetotoxicity) includes premature birth, prenatal death, stunting, histopathological effects, and transient biochemical or physiological changes. Behavioral teratogenicity, consisting of behavioral alterations or functional (e.g., motor, sensory) deficits resulting from *in utero* exposure, is considered in Section 12.4.3 of this chapter.

Teratogenicity of lead, at high exposure levels, has been demonstrated in rodents and birds, with some results suggesting a species-related specificity of certain gross teratogenic effects. Ferm and Carpenter (1967), as well as Ferm and Ferm (1971), reported increased embryonic resorption and malformation rates when various lead salts were administered i.v. to pregnant hamsters. Teratogenic effects were largely restricted to the tail region, including malformations of sacral and caudal vertebrae resulting in absent or stunted tails. Gale (1978) found the same effects, plus hydrocephalus, among six strains of hamsters and noted differences in susceptibility, suggesting a genetic component in lead-induced teratogenicity.

Zegarska et al. (1974) performed a study with rats injected with lead acetate at mid-gestation. They reported embryonic mortality and malformations. McClain and Becker (1975) subsequently administered lead nitrate i.v. to rats on one of days 8-17 of gestation, producing malformations and embryo- and fetotoxicity. Hackett et al. (1978, 1982a,b) also gave lead i.v. to rats and found malformations and high incidences of prenatal mortality. Minsker et al. (1982) gave lead i.v. to dams on day 17 of gestation and observed decreased birth weights as well as decreased weight and survival by postpartum day 7.

In another study, Miller et al. (1982) used oral doses of lead acetate up to 100 mg/kg given daily to rats before breeding and throughout pregnancy and found fetal stunting at the high dose, but no other effects. Maternal blood lead values ranged from 80 to 92 μg/dl prior to mating and from 53 to 92 μg/dl during pregnancy. Pretreatment and control blood lead levels averaged 6-10 μg/dl. Also, Wardell et al. (1982) gavaged rats daily with lead doses of up to 150 mg/kg from gestation day 6-18 and observed decreased prenatal survival at the high dose, but no malformations.

Ferm (1969) reported that teratogenic effects of i.v. lead in hamsters are potentiated in the presence of cadmium, leading to severe caudal dysplasia. This finding was duplicated by Hilbelink (1980). In addition to caudal malformations, lead appears to influence the morphology of the developing brain. For example, Murray et al. (1978) described a significant decrease in number of dendritic spines and observed a variety of morphological abnormalities of such spines in the parietal cortex of 30-day-old rat pups exposed to lead during gestation and nursing, during the postweaning period only, or during both periods. Morphometric analysis of rats transplacentally exposed to lead indicated that cellular organelles were altered as a function of dose and stage of development at exposure (Klein et al., 1978). These results indicate that morphologically apparent effects of lead on the brain could be produced by exposure during pregnancy alone, a question not addressed by Murray et al. (1978). See Section 12.4.3 for a discussion of other studies relating lead exposure to morphological and functional alterations in the CNS of developing animals.

12.6.2.2.4.2 Low-dose effects on the conceptus. There is a paucity of information regarding the teratogenicity and developmental toxicity of prolonged low-level lead exposure. Kimmel et al. (1980) exposed female rats chronically to lead acetate via drinking water (0.5, 5, 50, and 250 ppm) from weaning through mating, gestation, and lactation. They observed a decrease in fetal body length of female offspring at the high dose, and the female offspring from the 50 and 250 ppm groups weighed less at weaning and showed delays in physical development. Maternal toxicity was evident in the rats given 25 ppm or higher doses, corresponding to blood lead levels of 20 µg/dl or higher. Reiter et al. (1975) observed delays in the development of the nervous system in offspring exposed to 50 ppm lead throughout gestation and lactation. Whether these delays in development resulted from a direct effect of lead on the nervous system of the pups or reflect secondary changes (resulting from malnutrition, hormonal imbalance, etc.) is not clear. Whatever the mechanisms involved, these studies suggest that low-level, chronic exposure to lead may induce postnatal developmental delays.

12.6.2.2.4.3 Prenatal effects of organolead compounds. In an initial study of the effects of organolead compounds in animals, McClain and Becker (1972) treated rats orally with 7.5-30 mg/kg tetraethyl lead, 40-160 mg/kg tetramethyl lead, or 15-38 mg/kg trimethyl lead chloride, given in three divided doses on gestation days 9-11 or 12-14. The last compound was also given intravenously at doses of 20-40 mg/kg on one of days 8-15 of pregnancy. The highest dose of each agent resulted in maternal death, while lower doses caused maternal toxicity. At all dose levels, fetuses from dams given multiple treatment weighed less than controls. Single treatments at the highest doses tended to have similar effects. In some cases delayed ossification was observed. In addition, direct intra-amniotic injection of trimethyl lead chloride at levels up to 100 µg per fetus caused increasing fetal mortality.

Kennedy et al. (1975) administered tetraethyl lead by gavage to mice and rats during the period of organogenesis at dose levels up to 10 mg/kg. Maternal toxicity, prenatal mortality, and developmental retardation were noted at the highest doses in both species, although maternal treatment was discontinued after only three days due to excessive toxicity. In a subsequent study involving alkyl lead, Odenbro and Kihlström (1977) treated female mice orally with triethyl lead at doses of up to 3.0 mg/kg per day on days 3-5 following mating. The highest treatment levels resulted in decreased pregnancy rates, while at 1.5 mg/kg, lower implantation rates were seen. In order to elucidate the mechanism of implantation failure in organolead-intoxicated mice, Odenbro et al. (1982) measured plasma sex steroid levels in mice five days after mating. Levels of both estradiol and progesterone, but not estrone, were decreased following intraperitoneal triethyl lead chloride on days three and four of gestation. Such results suggest a hormonal mechanism for blockage of implantation, a finding also suggested for inorganic lead (Wide, 1980; Jacquet et al., 1977a). In an attempt to elucidate the mechanism by which organolead compounds decrease fetal growth, Kihlstrom and Odenbro (1983) treated guinea pigs with i.p. triethyl lead chloride. They observed reduced placental transfer of alpha-amino isobutyrate following doses of 2.5 mg/kg, but no effect was seen at 1.0 mg/kg.

12.6.2.2.4.4 Effects of lead on fetal physiology and metabolism. Biochemical indicators of developmental toxicity have been the subject of a number of investigations, as possible indicators of subtle prenatal effects. Hubermont et al. (1976) exposed female rats to lead in drinking water before mating, during pregnancy, and after delivery. In the highest exposure group (10 ppm), maternal and offspring blood lead values were elevated and approached 68 and 42 µg/dl, respectively. Inhibition of ALA-D and elevation of free tissue porphyrins were also noted in the newborns. Maternal diets containing up to 0.5 percent lead were associated with increased fetal porphyrins and decreased ALA-D activity by Jacquet et al. (1977a). Fetuses in the high-dose group had decreased weights, but no data were presented on maternal weight gain or food consumption (which could have influenced fetal weight).

Fetal effects were also investigated by Hayashi (1983a,b), who reported that lead levels as low as 5 ppm in the drinking water of rats for the first 18-21 days of pregnancy resulted in decreased ALA-D activity in the fetal erythrocytes. Fetal hepatic ALA-D activity was increased in the lead-treated groups, while hematocrit and hemoglobin concentrations were decreased by day 21. Fetal blood leads were 27 ± 16 and 19 ± 10 µg/dl in the 18- and 21-day groups, respectively.

In the only inhalation exposure study (Prigge and Greve, 1977), rats were exposed throughout gestation to an aerosol containing 1, 3, or 10 mg Pb/m^3 or to a combination of 3 mg Pb/m^3 and 500 ppm carbon monoxide (CO). Both maternal and fetal ALA-D activities were strongly inhibited by lead exposure in a dose-related manner. In the presence of lead plus CO, however, fetal (but not maternal) ALA-D activity was higher than in the group given lead alone, possibly due to the increase in total ALA-D seen in the CO-plus-lead treated fetuses. Fetal body weight and hematocrit were decreased in the high-dose lead group, while maternal values were unchanged, thus suggesting that the fetuses were more sensitive to lead's effects than were the mothers. Granahan and Huber (1978) also reported decreased hematocrit, as well as reduced hemoglobin levels, in fetal rats from lead-intoxicated dams (1000 ppm in the diet throughout gestation).

Gerber and Maes (1978) fed pregnant mice diets containing up to one percent lead from day 7 to 18 of pregnancy and determined levels of heme synthesis. Incorporation of iron into fetal heme was inhibited, but glycine incorporation into heme and protein was unaffected. Gerber et al. (1978) also found that dietary lead given late in gestation resulted in diminished placental blood flow but did not decrease uptake of a non-metabolizable amino acid, alpha-amino isobutyrate. The authors could not determine whether lead-induced fetal growth retardation was due to placental insufficiency or to the previously described reduction in heme synthesis (Gerber and Maes, 1978). They did not mention the possibility that the treated mothers may have reduced their food consumption, resulting in a reduced nutrient supply to the fetus, regardless of fetal ability to absorb nutrients.

In another study where evidence of physiological changes was seen at low lead levels, rats were given 0, 5, 25, 100, or 500 ppm lead in their drinking water throughout gestation and lactation (Victery et al., 1983). Their offspring were tested at one month of age and plasma renin activity was found to be elevated at all dose levels, while renal renin concentrations were elevated at the two highest doses. The increases in plasma angiotensin II (AII) levels found in the offspring of rats treated with 100 and 500 ppm lead were partially inhibited when the one-month-old pups were anesthetized and subjected to a surgical procedure (laparotomy) prior to sampling. Such results suggest that exposure to relatively low lead levels during development and via nursing may enhance basal renin secretion in young rats, while at least at higher levels (the two low-dose groups were not tested for AII), such treatment tended to inhibit the response to renin-releasing conditions.

More recently, Wardell et al. (1982) exposed rat fetuses *in utero* to lead by gavaging their pregnant mothers with 150 mg/kg on gestation days 6-18. On day 19, fetal limb cartilage was tested for ability to synthesize protein, DNA, and proteoglycans, but no adverse effects were seen. Also, Talcott and Koller (1983) found no effect on the immune system of the offspring of mice exposed during gestation to 1000 ppm dietary lead with or without Aroclor 1254.

12.6.2.2.4.5 *Possible mechanisms of lead-induced teratogenesis*. The reasons for the localization of many of the gross teratogenic effects of lead are unknown at this time. Ferm and Ferm (1971) have suggested that the observed specificity could be explained by an interference with specific enzymatic events. Lead alters mitochondrial function and enhances or inhibits enzymes (see Section 12.2.1); any or all such effects could ultimately interfere with normal development. Similarly, inhibition of ALA production has been suggested as a mechanism of teratogenesis by Cole and Cole (1976), while Danielsson et al. (1983) have proposed that lead's teratogenic effects may be based in part on a functional oxygen deficiency in certain tissues due to an interference with fetal heme production.

In an attempt to study the mechanics of lead induction of sacral-tail region malformations, Carpenter and Ferm (1977) examined hamster embryos treated at mid-gestation during the critical stage for response to teratogens in this species. The initial effects were edema of the tail region of embryos 30 hours after maternal exposure, followed by blisters and hematomas. These events disrupted normal caudal development, presumably by mechanical displacement. The end results seen in surviving fetuses were missing, stunted, or malformed tails and anomalies of the lower spinal cord and adjacent vertebrae.

12.6.2.2.4.6 Maternal factors in lead-induced teratogenesis and fetotoxicity. Nutritional factors may also have a bearing on the prenatal toxicity of lead. Jacquet and Gerber (1979) reported increased mortality and defects in fetuses of mice given intraperitoneal injections of lead while consuming a calcium-deficient diet during gestation. In several treatment groups, lead-treated calcium-deficient mothers had low blood calcium levels, while controls on the same diet had normal values. It is not certain how meaningful these data are, however, as there was no clear dose-response relationship within diet groups. In fact, fetal weights were said to be significantly higher in two of the lead-treated groups (on the normal diet) than in the untreated controls. Another problem with the study was that litter numbers were small.

In a later study, Carpenter (1982) reported greater prenatal mortality and incidence of malformations in fetal hamsters from mothers given 0.05 or 0.1 percent lead acetate in their drinking water if the mothers were also on diets deficient in either calcium or iron. Numbers of litters per group were small, however, and the two lead dose groups were combined when the data were averaged, making the results difficult to interpret.

Another study on interactions of lead with other elements was done by Dilts and Ahokas (1979), who exposed rats to lead in their drinking water throughout gestation. Controls were pair-fed or fed ad libitum. Lead treatment was said to result in decreased fetal weight, and dietary zinc supplementation was claimed to be associated with a protective effect against fetal stunting. The data as presented do not allow the differentiation of effects due to maternal stress (e.g., decreased food consumption) from direct effects on the fetus. In addition, litter numbers were small, and some of the data were confusing. For example, a lead-treated and a pair-fed group had very similar litter sizes and total litter weights, but rather dissimilar average fetal weights; also, dividing live litter weight by live litter size does not give the authors' values for average fetal weight. Finally, no data were given on maternal or fetal lead or zinc levels. In a further report on apparently the same animals as above, Dilts and Ahokas (1980) found that lead inhibited cell division and decreased protein contents of the fetal placentas, eviscerated carcasses, and livers. Such lead-related effects were not influenced by maternal zinc supplementation.

12.6.2.3 Effects of Lead on Avian Species. The effects of lead on the reproduction and development of various avian species have been studied by a number of investigators, primarily because of interest in the effects of lead shot ingested by wildlife or in order to develop an avian embryo model for the experimental analysis of ontogenetic processes. The relevance of such studies to the health effects of lead on humans is not clear. Consequently, these studies are not discussed further here.

12.6.3 Summary

The most clear-cut data described in this section on reproduction and development are derived from studies employing high lead doses in laboratory animals. There is still a need for more critical research to evaluate the possible subtle toxic effects of lead on the fetus, using biochemical, ultrastructural, or behavioral endpoints. An exhaustive evaluation of lead-associated changes in offspring should include consideration of possible effects due to paternal lead burden as well. Neonatal lead intake via consumption of milk from lead-exposed mothers may also be a factor at times. Moreover, it must be recognized that lead's effects on reproduction may be exacerbated by other environmental factors (e.g., dietary influences, maternal hyperthermia, hypoxia, and co-exposure to other toxins).

There are currently no reliable data pointing to adverse effects in human offspring following lead exposure of fathers *per se*. Early studies of pregnant women exposed to high levels of lead indicated toxic, but not teratogenic, effects on the conceptus. Unfortunately, the collective human data regarding lead's effects on reproduction or *in utero* development currently do not lend themselves to accurate estimation of exposure-effect or no-effect levels. This is particularly true regarding lead effects on reproductive performance in women, which have not been well documented at low exposure levels. Still, prudence would argue for avoidance of lead exposures resulting in blood lead levels exceeding 25-30 μg/dl in pregnant women or women of child-bearing age in general, given the equilibration between maternal and fetal blood lead concentrations that occurs and the growing evidence for deleterious effects in young children as blood lead levels approach or exceed 25-30 μg/dl. Industrial exposure of men to lead at levels resulting in blood lead values of 40-50 μg/dl also appear to result in altered testicular function.

The paucity of human exposure data forces an examination of the animal studies for indications of threshold levels for effects of lead on the conceptus. It must be noted that the animal data are almost entirely derived from rodents. Based on these rodent data, it seems likely that fetotoxic effects have occurred in animals at chronic exposures to 600-800 ppm inorganic lead in the diet. Subtle effects appear to have been observed at 5-10 ppm in the drinking water, while effects of inhaled lead have been seen at levels of 10 mg/m^3. With multiple exposure by gavage, the lowest observed effect level is 64 mg/kg per day, and for exposure via injection, acute doses of 10-16 mg/kg appear effective. Since humans are most likely to be exposed to lead in their diet, air, or water, the data from other routes of exposure are of less value in estimating harmful exposures. Indeed, it appears that teratogenic effects occur in experimental animals only when the maternal dose is given by injection.

Although human and animal responses may be dissimilar, the animal evidence does document a variety of effects of lead exposure on reproduction and development. Measured or apparent changes in production of or response to reproductive hormones, toxic effects on the gonads, and toxic or teratogenic effects on the conceptus have all been reported. The animal data also suggest subtle effects on such parameters as metabolism and cell structure that should be monitored in human populations. Well-designed human epidemiological studies involving large numbers of subjects are still needed. Such data could clarify the relationship of exposure levels and durations to blood lead values associated with significant effects and are needed for estimation of no-effect levels. (Recent studies, most of which are prospective epidemiological investigations, on the relationship between relatively low-level lead exposure and effects on fetal and child development, along with supporting experimental evidence on possible underlying mechanisms, are reviewed in an Addendum to this document.)

12.7 GENOTOXIC AND CARCINOGENIC EFFECTS OF LEAD

12.7.1 Introduction

Potential carcinogenic, genotoxic (referring to alteration in structure or metabolism of DNA), and mutagenic roles of lead are considered here. Epidemiological studies of occupationally exposed populations are considered first. Such studies investigate possible associations of lead with induction of human neoplasia and are important because they assess the incidence of disease in humans under actual ambient exposure conditions. However, such studies have many limitations that make it difficult to assess the carcinogenic activity of any specific agent. These include general problems in accurately determining the amount and nature of exposure to a particular chemical agent; in the absence of adequate exposure data it is difficult to determine whether each individual in a population was equally exposed to the agent in question. It is also often difficult to assess other factors, such as exposure to carcinogens in the diet, and to control for confounding variables that may have contributed to the incidence of any neoplasms. These factors tend to obscure the effect of lead alone. Also, in an occupational setting a worker is often exposed to various chemical compounds, making it more difficult to assess epidemiologically the injurious effect resulting specifically from exposure to one, such as lead.

A second approach considered here examines the ability of specific lead compounds to induce tumors in experimental animals. The advantage of these studies over epidemiological investigations is that a specific lead compound, its mode of administration, and level of exposure can be well defined and controlled. Additionally, many experimental procedures can be performed on animals that for ethical reasons cannot be performed on humans, thereby allowing a better understanding of the course of chemically induced injury. For example, animals may be sacrificed and necropsies performed at any desired time during the study. Factors such as diet and exposure to other environmental conditions can be well controlled, and genetic variability can be minimized by use of well established and characterized animal lines. One problem with animal studies is the difficulty of extrapolating such data to humans. However, this drawback is perhaps more important in assessing the toxicity of organic chemicals than in assessing inorganic agents, because the injury induced by many organic agents is highly dependent upon reactive intermediates formed *in vivo* by enzymatic action (e.g., microsomal enzymes) upon the parent compound. In addition, both qualitative and quantitative differences between the metabolic capabilities of humans and experimental animals have been documented (Neal, 1980). With inorganic compounds of lead, however, the element of interest undergoes little alteration *in vivo* and, therefore, the ultimate toxic agent is less likely to differ between experimental animals and humans (Costa, 1980). The carcinogenic action of most organic chemicals is dependent upon activation of a parent pro-carcinogen, whereas most metallic carcinogens undergo little alteration *in vivo* to produce their oncogenic effects (Costa, 1980).

A third approach discussed below is *in vitro* studies. Animal carcinogen bioassays are currently the preferred means for assessing carcinogenic activity but they are extremely expensive and time consuming. As a result, much effort has been directed toward developing suitable *in vitro* tests to complement *in vivo* animal studies for the evaluation of the potential oncogenicity of chemicals. The cell transformation assay has as its endpoint neoplastic transformation of mammalian cells and is the most suitable *in vitro* system because it examines cellular events closely related to carcinogenesis (Heck and Costa, 1982a). A general problem with this assay system, which is less troublesome with reference to metal compounds, is that it employs fibroblastic cells in culture, which lack many *in vivo* metabolic systems. Since lead is not extensively metabolized *in vivo*, addition of liver microsomal extracts (which has been attempted in this and similar systems) is not necessary to generate the ultimate carcinogen(s) from this metal (see above). However, if other indirect factors are involved with lead carcinogenesis *in vivo*, then these might be absent in such culture systems (e.g., specific lead-binding proteins that direct lead interactions *in vivo* with oncogenically relevant sites). There are also technical problems related to the culturing of primary cells and difficulties with the final microscopic evaluation of morphological transformations, which are prone to some subjectivity. However, if the assay is performed properly it can be very reliable and reproducible. Modifications of this assay system (i.e., exposure of pregnant hamsters to a test chemical followed by culturing and examination of embryonic cells for transplacentally induced transformation) are available for evaluation of *in vivo* metabolic influences, provided that the test agent is transported to the fetus. Additionally, cryopreservation of primary cultures isolated from the same litter of embryos can control for variation in cell populations exposed to test chemicals and give more reproducible responses in replicate experiments (Pienta, 1980). A potential advantage of the cell transformation assay system is the possibility that cultured human cells can be transformed *in vitro*. Despite numerous attempts, however, no reproducible human-cell transformation system has yet been sucessfully established which has been evaluated with a number of different chemicals of defined carcinogenic activity.

Numerous processes have been closely linked with oncogenic development, and specific assay systems that utilize events linked mechanistically with cancer as an endpoint have been developed to probe whether a chemical agent can affect any of these events. These systems include assays for mutations, chromosomal aberrations, development of micronuclei, enhancement of sister chromatid exchange, effects on DNA structure, and effects on DNA and RNA polymerase. These assay systems have been used to examine the genotoxicity of lead and facilitate the assessment of possible lead carcinogenicity. Chromosomal aberration studies are useful because human lymphocytes cultured from individuals after exposure to lead allow evaluation of genotoxic activity that occurred under the influence of an in vivo metabolic system. Such studies are discussed below in relationship to genotoxic effects of lead. However, a neoplastic change does not necessarily result, and evaluations of some less conspicuous types of chromosomal aberrations are somewhat subjective since microscopy is exclusively utilized in the final analyses. Nevertheless, it is reasonable to assume that if an agent produces chromosomal aberrations it may have potential carcinogenic activity. Many carcinogens are also mutagenic, and this fact, combined with the low cost and ease with which bacterial mutation assays can be performed, has resulted in wide use of these systems in determining potential carcinogenicity of chemicals. Mutation assays can also be performed with eukaryotic cells and several studies are discussed below that examined the mutagenic role of lead in these systems. However, in bacterial systems such as the Ames test, metal compounds with known human carcinogenic activity are generally negative and, therefore, this system is not useful for determining the potential oncogenicity of lead. Similarly, even in eukaryotic systems, metals with known human cancer-causing activity do not produce consistent mutagenic responses. Reasons for this lack of mutagenic effect remain unclear, and it appears that mutagenicity studies of lead cannot be weighed heavily in assessing its genotoxicity.

Other test systems that probe for effects of chemical agents on DNA structure may be useful in assessing the genotoxic potential of lead. Sister chromatid exchange represents the normal movement of DNA in the genome and enhancement of this process by potentially carcinogenic agents is a sensitive indicator of genotoxicity (Sandberg, 1982). Numerous recently developed techniques can be used to assess DNA damage induced by chemical carcinogens. One of the most sensitive is alkaline elution (Kohn et al., 1981), which may be used to study DNA lesions produced in vivo or in cell culture. This technique can measure DNA strand breaks or crosslinks in DNA, as well as repair of these lesions, but the toxicity of lead compounds has not been studied with this technique. Assessment of the induction of DNA repair represents one of the most sensitive techniques for probing genotoxic effects. The reason for this is that the other procedures measure DNA lesions that have persisted either because they were not recognized by repair enzymes or because their number was sufficiently great to saturate DNA repair systems. Measurement of DNA repair activation is still possible even if the DNA lesion has been repaired, but effects of lead compounds on DNA repair have not been studied. There are a few isolated experiments within publications that examined the ability of lead compounds to induce DNA damage, but this line of investigation requires further work. There are some well-conducted in vitro studies of the effect of lead along with other water soluble metals on isolated DNA and RNA polymerases, which suggest mutagenic mechanisms occurring in intact cells. The ability of lead to affect the transcription of DNA and RNA merits concern in regard to its potential oncogenic and mutagenic properties.

12.7.2 Carcinogenesis Studies of Lead and its Compounds

12.7.2.1 Human Epidemiological Studies. Epidemiological studies of industrial workers, where the potential for lead exposure is usually greater than for a "normal population," have been conducted to evaluate the role of lead in the induction of human neoplasia (Cooper, 1976, 1981; Cooper and Gaffey, 1975; Chruściel, 1975; Dingwall-Fordyce and Lane, 1963; Lane, 1964; McMichael and Johnson, 1982; Neal et al., 1941; Nelson et al., 1982). In general, these studies made no attempt to consider types of lead compounds to which workers were exposed or to determine probable routes of exposure. Some information on specific lead compounds encountered in the various occupational settings, along with probable exposure routes, would have made the studies more interpretable and useful. As noted in Chapter 3, with the exception of lead nitrate and lead acetate, many inorganic lead salts are relatively water insoluble. If exposure occurred by ingestion, the ability of water-insoluble lead salts (e.g., lead oxide and lead sulfide) to dissolve in the gastrointestinal tract may contribute to understanding of their ultimate systemic effects in comparison to their local actions in the gastrointestinal tract. Factors such as particle size are also important in the dissolution of any water insoluble compounds in the gastrointestinal system (Mahaffey, 1983). When considering other routes of exposure (e.g., inhalation), the water solubility of the lead compound in question, as well as the particle size, are extremely important, both in terms of systemic absorption and contained injury in the immediate locus of the retained particle (see Chapter 10). A hypothetical example is the inhalation of an aerosol of lead oxide versus a water soluble lead salt such as lead acetate. Lead oxide particles having a diameter of <5 μm would tend to deposit in the lung and remain in contact with cells there until they dissolved, while soluble lead salts would dissipate systemically at a much more rapid rate. Therefore, in the case of inhaled particulate compounds, localized exposure to lead might produce injury primarily in respiratory tissue, whereas with soluble salts, systemic (i.e., CNS, kidney, and erythropoietic) effects might predominate.

The studies of Cooper and Gaffey (1975) and Cooper (1976, 1981) examined the incidence of cancer in a large population of industrial workers exposed to lead. Two groups of individuals were identified as the lead-exposed population under consideration: smelter workers from six lead production facilities and battery plant workers (Cooper and Gaffey, 1975). The authors reported (see Table 12-17) that total mortality from cancer was higher in lead smelter workers than in a control population in two ways: (1) the difference between observed and expected values for the types of malignancies reported; and (2) the standardized mortality ratio (SMR), which, by comparison to a control population, indicates a greater than "normal" (but not necessarily statistically significant) response if it is in excess of 100 percent. These studies report not only an excess of all forms of cancer in smelter workers but also a greater level of cancer in the respiratory and digestive systems in both battery plant and smelter workers.

TABLE 12-17. EXPECTED AND OBSERVED DEATHS AND STANDARDIZED MORTALITY RATIOS FOR MALIGNANT NEOPLASMS FROM JAN. 1, 1947 TO DEC. 31, 1979 FOR LEAD SMELTER AND BATTERY PLANT WORKERS

Causes of death (ICD[a] Code)	Smelters			Battery Plant		
	Observed	Expected	SMR[b]	Observed	Expected	SMR[c]
All malignant neoplasms (140-205)	69	54.95	133	186	180.34	111
Buccal cavity & pharynx (140-148)	0	1.89	--	6	6.02	107
Digestive organs peritoneum (150-159)	25	17.63	150	70	61.48	123
Respiratory system (160-164)	22	15.76	148	61	49.51	132
Genital organs (170-179)	4	4.15	101	8	18.57	46
Urinary organs (180-181)	5	2.95	179	5	10.33	52
Leukemia (204)	2	2.40	88	6	7.30	88
Lymphosarcoma, lymphatic, and hematopoietic (200-203, 205)	3	3.46	92	7	9.74	77
Other sites	8	6.71	126	23	17.39	142

[a] International Classification of Diseases.

[b] Correction of +5.55% applied for 18 missing death certificates; SMR = standardized mortality ratio.

[c] Correction of +7.52% applied for 71 missing death certificates.

Source: Cooper and Gaffey (1975).

The incidence of urinary system cancer was also elevated in the smelter workers (but not in the battery plant workers), although the number of individuals who died from this neoplasm was very small. As the table indicates, death from neoplasm at other sites was also elevated compared with a normal population, but these results were not discussed in Cooper and Gaffey's (1975) report, since these elevated incidences of cancer were not statistically significant by their analysis.

Kang et al. (1980) examined the Cooper and Gaffey (1975) report and noted an error in the statistical equation used to assess the significance of excess cancer mortality. Table 12-18, from Kang et al. (1980) shows results based on what they claimed was a corrected form of the statistical equation previously used by Cooper and Gaffey (1975); it also employed another statistical test claimed to be more appropriate. Statistical significance was observed in every category listed with the exception of battery plant workers, whose deaths from all forms of neoplasia were not different from a control population. Gaffey (1980), in responding to the letter of Kang et al. (1980), indicated that a typographical error had been made in the equation printed in their publication (Cooper and Gaffey, 1975) but that the correct equation had actually been used in assessing the statistical significance of their data.

Cooper and Gaffey (1975) did not discuss types of lead compounds that these workers may have been exposed to in smelting operations but workers thus employed likely ingested or

TABLE 12-18. EXPECTED AND OBSERVED DEATHS RESULTING FROM SPECIFIED MALIGNANT NEOPLASMS FOR LEAD SMELTER AND BATTERY PLANT WORKERS AND LEVELS OF SIGNIFICANCE BY TYPE OF STATISTICAL ANALYSIS ACCORDING TO ONE-TAILED TESTS

Causes of death (ICD[a] code)	Number of deaths		SMR[b]	Probability		
	Observed	Expected		Poisson[c]	This analysis[d]	Cooper and Gaffey[e]
Lead smelter workers:						
All malignant neoplasms (140-205)	69	54.95	133	<0.02	<0.01	<0.02
Cancer of the digestive organs, peritoneum (250-159)	25	17.63	150	<0.03	<0.02	<0.05
Cancer of the respiratory system (160-164)	22	15.76	148	<0.05	<0.03	>0.05
Battery plant workers:						
All malignant neoplasms (140-205)	186	180.34	111	>0.05	>0.05	>0.05
Cancer of the digestive organs, peritoneum (150-159)	70	61.48	123	<0.05	<0.04	>0.05
Cancer of the respiratory system (160-164)	61	49.51	132	<0.03	<0.02	<0.03

[a] International Classification of Diseases.

[b] Standardized mortality ratios (SMRs) were corrected by Cooper and Gaffey for missing death certificates under the assumption that distribution of causes of death was the same in missing certificates as in those that were obtained.

[c] Observed deaths were recalculated as follows: adjusted observed deaths = (given SMR/100) x expected deaths.

[d] Given $z = (\mathrm{SMR} - 100)\sqrt{\mathrm{expected}}/100$.

[e] Given $z = (\mathrm{SMR} - 100)/\sqrt{100 \times \mathrm{SMR}/\mathrm{expected}}$.

Source: Kang et al. (1980).

inhaled oxides and sulfides of lead. Since these and other lead compounds produced in the industrial setting are not readily soluble in water it could be that the cancers arising in respiratory or gastrointestinal systems were caused by exposure to water-insoluble lead compounds. Although the Cooper and Gaffey (1975) study had a large sample (7032), only 2275 of the workers (32.4 percent) were employed when plants monitored urinary lead. Urinary lead values were available for only 9.7 percent of the 1356 deceased employees on whom the cancer mortality data were based. Only 23 (2 percent) of the 1356 decedents had blood lead levels measured. Cooper and Gaffey (1975) did report some average urinary and blood lead levels, where 10 or more urine or at least three blood samples were taken (viz., battery plant workers: urine lead = 129 μg/l, blood lead = 67 μg/dl; smelter workers: urine lead = 73 μg/l, blood lead = 79.7 μg/dl). Cooper (1976) noted that these workers were potentially exposed to other materials, including arsenic, cadmium, and sulfur dioxide, although no data on such exposures were reported. In these and other epidemiological studies in which selection of subjects for monitoring exposure to an agent such as lead is left to company discretion, it is possible that individual subjects are monitored primarily on the basis of obvious signs of lead exposure, while other individuals who show no symptoms of lead poisoning would not be monitored (Cooper and Gaffey, 1975). It is also not clear from these studies when the lead levels were measured, although the timing of measurement would make little difference since no attempt was made to match an individual's lead exposure to any disease process.

In a follow-up study of the same population of workers, Cooper (1981) concluded that lead had no significant role in the induction of neoplasia. However, he did report SMRs of 149 percent and 125 percent for all types of malignant neoplasms in lead battery plant workers with <10 or >10 years of employment, respectively (Cooper, 1981). In battery workers employed for 10 years or more there was an unusually high incidence of cancer listed as "other site" tumors (SMR = 229 percent; expected = 4.85, observed = 16) (Cooper, 1981, Table 13). Respiratory cancers were elevated in the battery plant workers employed for less than 10 years (SMR = 172 percent). Similarly, in workers involved with lead production facilities for more than 10 years the SMR was 151 percent.

An analysis of data for a more carefully selected subset of the same population (6819 workers versus 7032 originally) for the period 1947-1980 was recently reported by Cooper (1985). Deaths due to malignant neoplasms were elevated in both cohorts of workers (SMRs = 113 percent), a significant excess in battery workers but not in smelter workers because of a smaller number of cases. Most of these deaths occurred prior to 1971, which accounts for the lack of such findings in Cooper's (1981) analysis of 1971-1975 data. Consistent with earlier findings, the primary tumor sites were the gastrointestinal tract and the lung. Cooper (1981, 1985) noted that the lack of information on smoking histories made interpretation of the respiratory cancers problematic. However, the association of lead exposure with gastric cancer is consistent with findings of Sheffet et al. (1982), who reported an increased, albeit nonsignificant, incidence of stomach cancer in workers exposed to lead chromate. Cooper (1985) suggested that high local concentrations of ingested lead could have a co-carcinogenic effect, particularly in those whose dietary or alcohol intake patterns predispose them to higher-than-average gastric cancer rates. As noted by Cooper, further study of the possible association between lead and gastric cancer seems advisable. At present, however, without better documentation of lead exposure histories, it is difficult to assess the degree of lead's contribution to the above findings.

A recent study (McMichael and Johnson, 1982) examined the historical incidence of cancers in a population of smelter workers diagnosed as having lead poisoning. The incidence of cancer in a relatively small group of 241 workers was compared with 695 deceased employees from the same company. The control group had been employed during approximately the same period and was asserted to be free from lead exposure, although there were no data to indicate lead levels in either the control or the experimental group. Based upon diagnoses of lead poisoning made in the 1920s and 1930s for a majority of the deaths, the authors concluded that there was a considerably lower incidence of cancer in lead-poisoned workers (McMichael and Johnson, 1982). However, there is no indication of how lead poisoning was diagnosed. It is difficult to draw any conclusions from this study with regard to the role of lead in human neoplasia.

Davies (1984) found that workers exposed to both lead and zinc chromate in English pigment factories showed a significantly increased incidence of lung cancer mortality after at least one year of medium or high exposure. However, lung cancer mortality was normal in workers exposed only to lead chromate, thus suggesting that zinc rather than lead chromate, was the more significant risk factor.

Another recent epidemiological study (Selevan et al., 1984) has noted increased mortality from renal cancer in a group of lead smelter workers. The SMR for deaths from renal cancer was 204 percent for the entire cohort, although this excess mortality was not statistically significant and only represented 6 cases. However, of interest is the fact that the renal cancers observed in humans in this study matched the types of cancers induced in experimental animals by lead. This study also analyzed the number of deaths associated with high lead exposure in combination with other contaminants (i.e., cadmium, zinc, and arsenic) as well as those deaths associated predominantly with high lead exposure alone. The SMR for deaths from renal cancer in the high lead exposure areas only was 301 percent. Similarly, deaths from cancers of the urinary organs had an SMR of 199 percent in the high-lead-only group. These results suggest that lead exposure could be associated with an increased incidence of renal cancer in humans, but in the absence of statistical significance and corroboration by other epidemiological studies, this finding should be interpreted with caution.

Two case studies have also suggested an association of lead exposure with renal cancer (Baker et al., 1980; Lilis, 1981). The relatively high degree of lead exposure in these two case reports was well documented by symptoms of lead intoxication and by measurements of blood lead and erythrocyte protoporphyrin. Furthermore, Baker et al. (1980) found a relatively high concentration of lead (~2.5 μg/g) in the patient's tumor as well as certain histologic similarities to lead-induced neoplasms in animal kidneys (e.g., swollen mitochondria, numerous dense lysosomes, and some amphophilic intranuclear inclusion bodies in epithelial cells adjacent to the proximal convoluted tubules). The generally sparse presence of intranuclear inclusion bodies in the patient's kidney was attributed to his use of oral penicillamine three to four weeks prior to being examined.

Conclusions regarding the ability of lead to induce human neoplasia must await further epidemiological studies in which other factors that may contribute to the observed effects are well controlled for and the disease process is assessed in individuals with well documented exposure histories. Little can now be reliably concluded from available epidemiological studies.

12.7.2.2 Induction of Tumors in Experimental Animals. As discussed in the preceding sections, it is difficult to obtain conclusive evidence of the carcinogenic potential of an agent using only epidemiological studies. Experiments testing the ability of lead to cause cancer in experimental animals are an essential aspect of understanding its oncogenicity in humans. However, a proper lifetime animal feeding study to assess the carcinogenic potential of lead following National Cancer Institute guidelines (Sontag et al., 1976) has not been conducted. The cost of such studies exceeds $1 million; consequently they are limited only to those agents in which sufficient evidence based upon *in vitro* or epidemiological studies warrants such an undertaking. The literature on lead carcinogenesis contains many smaller studies where only one or two doses were employed and where toxicological monitoring of experimental animals exposed to lead was generally absent. Some of these studies are summarized in Table 12-19 (see also Section 12.8.2.2). Most mainly serve to illustrate that numerous different laboratories have induced renal tumors in rats by feeding them diets containing 0.1 or 1.0 percent lead acetate. In some cases, other lead formulations were tested, but the dosage selection was not based upon lethal dose values. In most cases, only one dose level was used. Another problem with many of these studies was that the actual concentrations of lead administered and internal body burdens achieved were not measured. Some of these studies are discussed very briefly; others are omitted entirely because they contribute little to our understanding of lead carcinogenesis.

Administration of 1.0 percent lead acetate (10,000 ppm) resulted in kidney damage and a high incidence of mortality in most of the studies in Table 12-19. However, kidney tumors were also evident at lower dosages (e.g., 0.1 percent lead acetate in the diet), which produced less mortality among the test animals. As discussed in Section 12.6, renal damage is one of the primary toxic effects of lead. At 0.1 percent lead acetate (1000 ppm) in the diet, the concentration of lead measured in the kidney was 30 μg/g while 1 percent lead acetate resulted in 300 μg/g of lead in the kidneys of necropsied animals (Azar et al., 1973). In most of the studies with rats fed 0.1 or 1.0 percent lead in the diet, the incidence of kidney tumors increased between the lower and higher dosage, suggesting a relationship between the deposition of lead in the kidney and the carcinogenic response. Renal tumors were also induced in mice at the 0.1 percent oral dosage of lead subacetate but not in hamsters that were similarly exposed to this agent (Table 12-19).

TABLE 12-19. EXAMPLES OF STUDIES ON THE INCIDENCE OF TUMORS IN EXPERIMENTAL ANIMALS EXPOSED TO LEAD COMPOUNDS

Species	Pb compound	Dose and mode*	Incidence (and type) of neoplasms	Reference
Rat	Pb phosphate	120-680 mg (total dose s.c.)	19/29 (renal tumors)	Zollinger (1953)
Rat	Pb acetate	1% (in diet)	15/16 (kidney tumors) 14/16 (renal carcinomas)	Boyland et al. (1962)
Rat	Pb subacetate	0.1% and 1.0% (in diet)	11/32 (renal tumors) 13/24 (renal tumors)	Van Esch et al. (1962)
Mouse	Pb naphthenate	20% in benzene (dermal 1-2 times weekly)	5/59 (renal neoplasms) (no control with benzene)	Baldwin et al. (1964)
Rat	Pb phosphate	1.3 g (total dosage s.c.)	29/80 (renal tumors)	Balo et al. (1965)
Rat	Pb subacetate	0.5 - 1% (in diet)	14/24 (renal tumors)	Hass et al. (1967)
Rat	Pb subacetate	1% (in diet)	31/40 (renal tumors)	Mao and Molnar (1967)
Mouse	Tetraethyl lead in tricaprylin	0.6 mg (s.c.) 4 doses between birth and 21 days	5/41 (lymphomas) in females, 1/26 in males, and 1/39 in controls	Epstein and Mantel (1968)
Rat	Pb acetate	3 mg/day for 2 months; 4 mg/day for 16 months (p.o.)	72/126 (renal tumors) 23/94 males (testicular [Leydig cell] tumors)	Zawirska and Medraś (1968)
Hamster	Pb subacetate	1.0% (in 0.5% diet)	No significant incidence of renal neoplasms	Van Esch and Kroes (1969)
Mouse	Pb subacetate	0.1% and 1.0% (in diet)	7/25 (renal carcinomas) at 0.1%; substantial death at 1.0%	Van Esch and Kroes (1969)
Rat	Pb nitrate	25 g/l (in drinking water)	No significant incidence of tumors	Schroeder et al. (1970)
Rat	Pb acetate	3 mg/day (p.o.)	89/94 (renal, pituitary, cerebral gliomas, adrenal, thyroid, prostatic, mammary tumors)	Zawirska and Medraś, 1972

TABLE 12-19. (continued)

Species	Pb Compound	Dose and mode	Incidence (and type) of neoplasms	Reference
Rat	Pb acetate	0, 10, 50, 100, 1000, 2000 ppm (in diet) for 2 yr	No tumors 0-100 ppm; 5/50 (renal tumors) at 500 ppm; 10/20 at 1000 ppm; 16/20 males, 7/20 females at 2000 ppm	Azar et al. (1973)
Hamster	Pb oxide	1 mg (intratracheal) 10 times	0/30 without benzopyrene, 12/30 with benzopyrene (lung cancers)	Kobayashi and Okamoto (1974)
Rat	Pb chromate	8 mg (i.m.) for 9 monthly injections	Females: 2/25 lymphoma, 11/25 fibrosarcoma, 10/25 rhabdomyosarcoma, 1/25 osteogenic sarcoma Males: 3/25 fibrosarcoma, 7/25 rhabdomyosarcoma, 3/25 renal carcinoma	Furst et al. (1976)
Mouse	Pb chromate	3 mg (i.m.) for 4 monthly injections	Females only: 2/25 lymphoma, 3/25 lung carcinoma	Furst et al. (1976)
Rat	Pb acetate	0, 26, 2600 ppm (in drinking water) for 76 wk	81% (renal tumors) at 2600 ppm	Koller et al. (1985)

*s.c. = subcutaneous injection; p.o. = per os (gavage); i.m. = intramuscular injection.

Other lead compounds have also been tested in experimental animals, but in these studies only one or two dosages (generally quite high) were employed, making it difficult to assess the potential carcinogenic activity of lead compounds at relatively nontoxic concentrations. It is also difficult to assess the true toxicity caused by these agents, since properly designed toxicity studies were generally not performed in parallel with these cancer studies.

As shown in Table 12-19, lead nitrate produced no tumors in rats when given at very low concentrations, but lead phosphate administered subcutaneously at relatively high levels induced a high incidence of renal tumors in two studies. Lead powder administered orally resulted in lymphomas and leukemia; when given intramuscularly only one fibrosarcoma was produced in 50 animals. Lead naphthenate applied as a 20 percent solution in benzene two times each week for 12 months resulted in the development of four adenomas and one renal carcinoma in a group of 50 mice (Baldwin et al., 1964). However, in this study control mice were not painted with benzene. Tetraethyl lead at 0.6 mg given in four divided doses between birth and 21 days to female mice resulted in 5 of 36 surviving animals developing lymphomas, while 1 of 26 males treated similarly and 1 of 39 controls developed lymphomas (Epstein and Mantel, 1968).

Lead subacetate has also been tested in the mouse lung adenoma bioassay (Stoner et al., 1976). This assay measures the incidence of nodules forming in the lung of strain A/Strong mice following parenteral administration of various test agents. Nodule formation in the lung does not actually represent the induction of lung cancer but merely serves as a general measure of carcinogenic potency independent of lung tissue (Stoner et al., 1976). Lead subacetate was administered to mice at 150, 75, and 30 mg (total dose), which represented the maximum tolerated dose (MTD), 1/2 MTD, and 1/5 MTD, respectively, over a 30-week period using 15 separate i.p. injections (Stoner et al., 1976). Survivals at the three doses were 15/20 (MTD), 12/20 (1/2 MTD), and 17/20 (1/5 MTD), respectively, with 11/15, 5/12, and 6/17 survivors having lung nodules. Only at the highest doses was the incidence of nodules greater than in the untreated mice. However, these authors concluded that on a molar-dose basis lead subacetate was the most potent of all the metallic compounds examined. Injection of 0.13 mmol/kg lead subacetate was required to produce one lung tumor per mouse, indicating that this compound was about three times more potent than urethane (at 0.5 mmol/kg) and approximately 10 times more potent than nickelous acetate (at 1.15 mmol/kg). The mouse lung adenoma bioassay has been widely utilized for examining carcinogenic activity of chemical agents in experimental animals and is well recognized as a highly accurate test system for assessing potential carcinogenic hazards of metals and their compounds (Stoner et al., 1976). Recent studies utilizing the lung tumor bioassay in strain A mice have demonstrated that administration of magnesium or calcium acetates along with lead subacetate eliminated the tumorigenic activity of lead in this test system (Poirier et al., 1984). These results indicate that essential divalent metals can protect against the carcinogenic effects of lead. Lead oxide combined with benzopyrene administered intratracheally resulted in 11 adenomas and 1 adenocarcinoma in a group of 15 hamsters, while no lung neoplasias were observed in groups receiving benzopyrene or lead oxide alone (Kobayashi and Okamoto, 1974).

Administration of lead acetate to rats has been reported to produce other types of tumors, e.g., testicular, adrenal, thyroid, pituitary, prostate, lung (Zawirska and Medras, 1968), and cerebral gliomas (Oyasu et al., 1970). However, in other animal species, such as dogs (Azar et al., 1973; Fouts and Page, 1942) and hamsters (Van Esch and Kroes, 1969), lead acetate induced either no tumors or only kidney tumors (Table 12-19).

The above studies seem to implicate some lead compounds as carcinogens in experimental animals, but they were not designed to address the question of lead carcinogenesis in a definitive manner. In contrast, a study by Azar et al. (1973) examined the oncogenic potential of lead acetate at a number of doses and in addition monitored a number of toxicological parameters in the experimental animals. Azar et al. (1973) gave 0, 10, 50, 100, 500, 1000, and 2000 ppm dose levels of lead (as lead acetate) to rats during a two-year feeding study. Fifty rats of each sex were utilized at doses of 10-500 ppm, while 100 animals of each sex were used as controls. After the study was under way for a few months, a second 2-year feeding study was initiated using 20 animals of each sex in groups given doses of 0, 1000, or 2000 ppm. The study also included four male and four female beagle dogs at each dosage of lead ranging from 0 to 500 ppm in a 2-year feeding study. During this study, the clinical appearance and behavior of the animals were observed, and food consumption, growth, and mortality were recorded. Blood, urine, fecal, and tissue lead analyses were done periodically using atomic absorption spectrophotometry. A complete blood analysis was done periodically, including blood cell count, hemoglobin, hematocrit, stippled cell count, prothrombin time, alkaline phosphatase, urea nitrogen, glutamic-pyruvate transaminase, and albumin-to-globulin ratio. The activity of the enzyme delta-aminolevulinic acid dehydrase (ALA-D) in the blood and the excretion of its substrate, delta-aminolevulinic acid (ALA), in the urine were also determined. A thorough necropsy, including both gross and histologic examination, was performed on all animals.

Table 12-20 depicts the mortality and incidence of kidney tumors reported by Azar et al. (1973). At 500 ppm (0.05 percent) and above, male rats developed a significant number of renal tumors. Female rats did not develop tumors except when fed 2000 ppm lead acetate. Two out of four male dogs fed 500 ppm developed a slight degree of cytomegaly in the proximal convoluted tubule but did not develop any kidney tumors. The number of stippled erythrocytes increased at 10 ppm in the rats but not until 500 ppm in the dogs. ALA-D was decreased at 50 ppm in the rats but not until 100 ppm in the dogs. Hemoglobin and hematocrit, however, were not depressed in the rats until they received a dose of 1000 ppm lead. These results illustrate that the induction of kidney tumors coincides with moderate to severe toxicological doses of lead acetate, for it was at 500-1000 ppm lead in the diet that a significant increase in mortality occurred (see Table 12-20). At 1000 and 2000 ppm lead, 21-day-old weanling rats showed no tumors but did show histological changes in the kidney comparable to those seen in adults receiving 500 ppm or more lead in their diet. Also of interest from the Azar et al. (1973) study is the direct correlation obtained in dogs between blood lead level and kidney lead concentrations. A dietary lead level of 500 ppm produced a blood lead concentration of 80 µg/dl within 24 months, which corresponds to a level at which humans often show clinical signs of lead poisoning (see Section 12.4.1). The kidney lead concentration corresponding to this blood lead level was 2.5 µg/g (wet weight), while at 50 µg/dl in blood the kidney lead levels were 1.5 µg/g. Assuming similar pharmacokinetic distribution of lead in

TABLE 12-20. MORTALITY AND KIDNEY TUMORS IN RATS FED LEAD ACETATE FOR TWO YEARS

Nominal (actual)[a] concentration in ppm of Pb in diet	No. of rats of each sex	% mortality[b]		% Kidney tumors	
		Male	Female	Male	Female
0 (5)	100	37	34	0	0
10 (18)	50	36	30	0	0
50 (62)	50	36	28	0	0
100 (141)	50	36	28	0	0
500 (548)	50	52	36	10	0
0 (3)	20	50	35	0	0
1000 (1130)	20	50	50	50	0
2000 (2102)	20	80	35	80	35

[a] Measured concentration of lead in diet.

[b] Includes rats that either died or were sacrificed *in extremis*.

Source: Azar et al. (1973).

the dogs as in rats, it can be stated that chronic exposure to 500 ppm of dietary lead, producing prolonged elevation of blood lead to 80 µg/dl and resulting in a concentration of 2.5 µg/g in the kidney can cause elevation in the incidence of kidney tumors.

Animal carcinogenesis studies conducted with lead and its compounds are numerous; however, with the exception of the study by Azar et al. (1973), they do not provide much useful information. Most of the studies shown in Table 12-18 were conducted with only one lead compound in one animal species, the rat. In cases where other lead compounds were tested or where other animal species were used, only a single high dosage level was administered, and parameters of toxicity such as those monitored in the Azar et al. (1973) study were not measured. Although it is clear from these studies as a whole that lead is a carcinogen in experimental animals, until more investigations such as that of Azar et al. (1973) are conducted it is difficult to determine the relative carcinogenic potency of lead. There remains a need to test thoroughly the carcinogenic activity of lead compounds in experimental animals. These tests should include several modes of administration, many dosages spanning non-toxic as well as toxic levels, and several different lead compounds or at least a comparison of a relatively water-soluble form such as lead acetate with a less soluble form such as lead oxide.

12.7.2.3 Cell Transformation. Although cell transformation is an *in vitro* experimental system, its end point is a neoplastic change. There are two types of cell transformation assays: (1) those employing continuous cell lines; and (2) those employing cell cultures prepared from embryonic tissue. Use of continuous cell lines has the advantage of ease in preparation of the cell cultures, but these cells generally have some properties of a cancer cell. The absence of a few characteristics of a cancer cell in these continuous cell lines allows for an assay of cell transforming activity. End points include morphological transformation (ordered cell growth to disordered cell growth), ability to form colonies in soft agar-containing medium (a property characteristic of cancer cells), and ability of cells to form tumors when inoculated into experimental animals. Assays that utilize freshly isolated embryonic cells are generally preferred to those that use cell lines, because embryonic cells have not yet acquired any of the characteristics of a transformed cell. The cell transformation assay system has been utilized to examine the potential carcinogenic activity of a number of chemical agents, and the results seem to agree generally with the results of carcinogenesis tests using experimental animals. Cell transformation assays can be made quantitative by assessing the percentage of surviving colonies exhibiting morphological transformation. Verification of a neoplastic change can be accomplished by cloning these cells and testing their ability to form tumors in animals.

Lead acetate has been shown to induce morphological transformation in Syrian hamster embryo cells following a continuous exposure to 1 or 2.5 μg/ml of lead in culture medium for nine days (DiPaolo et al., 1978). The incidence of transformation increased from 0 percent in untreated cells to 2.0 and 6.0 percent of the surviving cells, respectively, following treatment with lead acetate. Morphologically transformed cells were capable of forming fibrosarcomas when cloned and administered to "nude" mice and Syrian hamsters, while no tumor growth resulted from similar inoculation with untreated cells (DiPaolo et al., 1978). In the same study, lead acetate was shown to enhance the incidence of simian adenovirus (SA-7) induction of Syrian hamster embryo cell transformation. Lead acetate also caused significant enhancement (~2- to 3- fold) at 100 and 200 μg/ml following three hours of exposure. In another study (Casto et al., 1979), lead oxide also enhanced SA-7 transformation of Syrian hamster embryo cells almost 4-fold at 50 μM following three hours of exposure (Casto et al., 1979). The significance of enhanced virally induced carcinogenesis in relationship to the carcinogenic potential of an agent is not well understood.

Morphological transformation induced by lead acetate was correlated with the ability of the transformed cells to form tumors in appropriate hosts (see above), indicating that a truly neoplastic change occurred in tissue culture. The induction of neoplastic transformation by lead acetate suggests that this agent is potentially carcinogenic at the cellular level. However, with in vitro systems such as the cell transformation assay it is essential to compare the effects of other, similar types of carcinogenic agents in order to evaluate the response and to determine the reliability of the assay. The incidence of transformation obtained with lead acetate was greater than the incidence following similar exposure to $NiCl_2$, but less than that produced by $CaCrO_4$ (Heck and Costa, 1982a). Both nickel and chromium have been implicated in the etiology of human cancer (Costa, 1980). These results thus suggest that lead acetate has effects similar to those caused by other metal carcinogens. In particular, the ability of lead acetate to induce neoplastic transformation in cells in a concentration-dependent manner is highly suggestive of potential carcinogenic activity. It should also be noted that lead acetate induced these transformations at concentrations that decreased cell survival by only 27 percent (DiPaolo et al., 1978). Further studies from other laboratories utilizing the cell transformation assay and other lead compounds are needed.

12.7.3 Genotoxicity of Lead

Since cancer is known to be a disease of altered gene expression, numerous studies have evaluated changes in DNA consequent to exposure to suspected carcinogenic agents. The general response associated with such alterations in regulation of DNA function has been called genotoxicity. Various assay systems developed to examine specific changes in DNA structure and function caused by carcinogenic agents include assays that evaluate chromosomal aberrations, sister chromatid exchange, mutagenicity, and functional and structural features of DNA metabolism. Lead's effects on these parameters are discussed below.

12.7.3.1 Chromosomal Aberrations. Two approaches have been used in the analysis of effects of lead on chromosomal structure. The first approach involves culturing lymphocytes either from humans exposed to lead or from experimental animals given a certain dosage of lead. The second approach involves exposing cultured lymphocytes directly to lead. For present purposes, emphasis will not be placed on the type of chromosomal aberration induced, since most of the available studies do not appear to associate any specific type of chromosomal aberration with lead exposure. Little is known of the significance of chromosomal aberrations in relationship to cancer, except that in a number of instances genetic diseases associated with chromosomal aberrations often enhance the probability of neoplastic disease. However, implicit in a morphologically distinct change in genetic structure is the possibility of an alteration in gene expression that represents a salient feature of neoplastic disease.

Contradictory reports exist regarding the induction of chromosomal aberrations in lymphocytes from humans exposed to lead (Tables 12-21 and 12-22). These studies have been grouped in two separate tables based upon their conclusions. Those studies reporting a positive effect of lead on chromosomal aberrations are indexed in Table 12-21, whereas studies reporting no association between lead exposure and chromosomal aberrations are indexed in Table 12-22. Unfortunately, these studies are difficult to thoroughly evaluate because of many unknown variables (e.g., absence of sufficient evidence of lead intoxication, no dose-response relationship, and absence of information regarding lymphocyte culture time). To illustrate, in a

TABLE 12-21. CYTOGENETIC INVESTIGATIONS OF CELLS FROM INDIVIDUALS EXPOSED TO LEAD: POSITIVE STUDIES

Number of exposed subjects	Number of controls	Cell culture time, hr	Lead level in blood, μg/dl, or urine, μg/ℓ	Exposed subjects	Type of damage	Remarks	References
8	14	?	62-89 (blood)	Workers in a lead oxide factory	Chromatid and chromosome	Increase in chromosomal damage correlated with increased ALA excretion	Schwanitz et al. (1970)
10	10	72	60-100 (blood)	Workers in a chemical factory	Chromatid gaps, breaks	No correlation with blood lead levels	Gath & Thiess (1972)
14	5	48	155-720 (urine)	Workers in a zinc plant, exposed to fumes & dust of cadmium, zinc & lead	Gaps, fragments, exchanges, dicentrics, rings	Thought to be caused by lead, not cadmium or zinc	Deknudt et al. (1973)
105	-	72	11.6-97.4 mean, 37.7 (blood)	Blast-furnace workers, metal grinders, scrap metal processers	"Structural abnormalities," gaps, breaks, hyperploidy	No correlation with ALA excretion or blood lead levels	Schwanitz et al. (1975)
11 (before and after exposure)	-	68-70	34-64 (blood)	Workers in a lead-acid battery plant and a lead foundry	Gaps, breaks, fragments	No correlation with ALA-D activity in red cells	Forni et al. (1976)
44	15	72	30-75 (blood)	Individuals in a lead oxide factory	Chromatid and chromosome aberrations	Positive correlation with length of exposure	Garza-Chapa et al. (1977)
23	20	48	44-95 (not given)	Lead-acid battery melters, tin workers	Dicentrics, rings, fragments	Factors other than lead exposure may be required for severe aberrations	Deknudt et al. (1977b)
20	20	46-48	53-100 (blood)	Ceramic, lead, & battery workers	Breaks, fragments	Positive correlation with blood lead levels	Sarto et al. (1978)
26 (4 low, 16 medium, 6 high exposure)	not given	72	22.5-65 (blood)	Smelter workers	Gaps, chromatid and chromosome aberrations	Positive correlation with blood lead levels	Nordenson et al. (1978)
12	18	48-72	24-49 (blood)	Electrical storage battery workers	Chromatid and chromosome aberrations		Forni et al. (1980)

Source: International Agency for Research on Cancer (1980), with modifications.

TABLE 12-22. CYTOGENETIC INVESTIGATIONS OF CELLS FROM INDIVIDUALS EXPOSED TO LEAD: NEGATIVE STUDIES

Number of exposed subjects	Number of controls	Cell culture time, hrs	Blood lead level, μg/dl	Exposed subjects	References
29	20	46-48	Not given, stated to be 20-30% higher than controls	Policemen "permanently in contact with high levels of automotive exhaust"	Bauchinger et al. (1972)
32	20	46-48	Range not given; highest level was 590 mg/l [sic]	Workers in lead manufacturing industry; 3 had acute lead intoxication	Schmid et al. (1972)
35	35	45-48	Control, <4; exposed, 4 - >12	Shipyard workers employed as "burners" cutting metal structures on ships	O'Riordan and Evans (1974)
24	15	48	19.3 (lead) 0.4 (cadmium)	Mixed exposure to zinc, lead, and cadmium in a zinc-smelting plant; significant increase in chromatid breaks and exchanges. Authors suggest that cadmium was the major cause of this damage	Bauchinger et al. (1976)
9	9	72	40 ± 5, for 7 weeks	Volunteers ingested capsules containing lead acetate	Bulsma & De France (1976)
30	20	48	Control, 11.8-13.2; exposed, 29-33	Children living near a lead smelter	Bauchinger et al. (1977)

Source: International Agency for Research on Cancer (1980).

number of the studies where lead exposure correlated with an increased incidence of chromosomal aberrations (Table 12-21), lymphocytes were cultured for 72 hours. Most cytogenetic studies have been conducted with a maximum culture time of 48 hours to avoid high background levels of chromosomal aberrations due to multiple cell divisions during culture. Therefore, it is possible that the positive effects of lead on chromosomal aberrations may require the longer culture period in order to be observed. Nonetheless, it is evident that in the negative studies, the blood lead concentration was generally lower than in the studies reporting a positive effect of lead on chromosomal aberrations, although in many of the latter instances blood lead levels indicated severe exposure. In some of these positive studies there was a correlation in the incidence of gaps, fragments, chromatid exchanges, and other chromosomal aberrations with blood lead levels (Sarto et al., 1978; Nordenson et al., 1978). However, as indicated in Table 12-21, in other studies there were no direct correlations between indices of lead exposure (i.e., ALA excretion) and numbers of chromosomal aberrations. Nutritional factors such as Ca^{2+} levels *in vivo* or *in vitro* are also important since it is possible that the effects of lead on cells may be antagonized by Ca^{2+} (Mahaffey, 1983; Poirier et al., 1984). As is usually the case in studies of human populations exposed to lead, exposure to other metals (zinc, cadmium, and copper) that may produce chromosomal aberrations was prevalent. These studies did not attempt to determine the specific lead compound to which the individuals were exposed.

In a more recent study by Forni et al. (1980), 18 healthy females occupationally exposed to lead were evaluated for chromosomal aberrations in their lymphocytes cultured for 48 or 72 hours. There were more aberrations at the 72-hour culture time compared with the 48-hour culture period in both control and lead-exposed groups, but this difference was not statistically significant. However, statistically significant differences from the 72-hour controls were noted in the 72-hour culture obtained from the lead exposed group. These results demonstrate that the extended 72-hour culture time results in increased chromosomal aberrations in the control lymphocytes and that the longer culture time was apparently necessary to detect the effects of lead on chromosomal structure. However, the blood lead levels in the exposed females ranged from 24 to 59 µg/dl, while control females had blood lead levels ranging from 22 to 37 µg/dl. The small effect of lead on chromosomal aberration may be due to the absence of sufficient differences in the extent of lead exposure. Additionally, many agents that induce chromosomal aberrations require extended time periods for the lesion to be expressed, as indicated above for lead.

Some studies have also been conducted on the direct effect of soluble lead salts on cultured human lymphocytes. In a study by Beek and Obe (1974), a longer (72-hr) culture time was used and lead acetate was found to induce chromosomal aberrations at 100 µM. Lead acetate had no effect on chromatid aberrations induced with X-rays or alkylating agents (Beek and Obe, 1975). In another study (Deknudt and Deminatti, 1978), lead acetate at 1 and 0.1 mM caused minimal chromosomal aberrations. Both cadmium chloride ($CdCl_2$) and zinc chloride ($ZnCl_2$) were more potent than lead acetate in causing these changes; however, both $CdCl_2$ and $ZnCl_2$ also displayed greater toxicity than lead acetate.

Chromosomal aberrations have been demonstrated in lymphocytes from cynomolgus monkeys treated chronically with lead acetate (6 mg/day, 6 days/week for 16 months), particularly when they were kept on a low-calcium diet (Deknudt et al., 1977a). These aberrations accompanying a low-Ca^{2+} diet were characterized by the authors as severe (chromatid exchanges, dispiralization, translocations, rings, and polycentric chromosomes). Similar results were observed in mice (Deknudt and Gerber, 1979). The effect of low calcium on chromosomal aberrations induced by lead could be due to interaction of Ca^{2+} and Pb^{2+} at the level of the chromosome (Mahaffey, 1983). Léonard and his coworkers found no effect of lead on the incidence of chromosomal aberrations in accidentally intoxicated cattle (Léonard et al., 1974) or in mice given 1 g Pb/l drinking water for 9 months (Léonard et al., 1973). However, Muro and Goyer (1969) found gaps and chromatid aberrations in bone marrow cells cultured for four days after isolation from mice that had been maintained on 1 percent dietary lead acetate for two weeks. Chromosomal loss has been reported (Ahlberg et al., 1972) in Drosophila exposed to triethyl lead (4 mg/l), but inorganic lead had no effect (Ramel, 1973). Lead acetate has also been shown to induce chromosomal aberrations in cultured cells other than lymphocytes, viz. Chinese hamster ovary cells (Bauchinger and Schmid, 1972).

These studies demonstrate that under certain conditions, lead compounds are capable of inducing chromosomal aberrations *in vivo* and in tissue cultures. The ability of lead to induce these chromosomal changes appears to be concentration-dependent and highly influenced by calcium levels. In lymphocytes isolated from patients or experimental animals, relatively long (72-hr) culture conditions are required for the abnormalities to be expressed, indicating a requirement for cellular processes (e.g., DNA repair) to interact with the hidden lead-induced DNA lesions to produce a morphologically manifested aberration.

12.7.3.2 *Sister Chromatid Exchange*. Sister chromatid exchange affords a means of visually assessing the normal movement of DNA in the genome. The sister chromatid exchange assay offers a very sensitive probe for the effects of genotoxic compounds on DNA rearrangement, as a number of chemicals with carcinogenic activity are capable of increasing these exchanges (Sandberg, 1982). The effect of lead on such movement has been examined in cultured lymphocytes (Beek and Obe, 1975), with no increase in exchanges observed at lead acetate concentrations of 0.01 mM. Two more recent studies have examined the effect of human lead exposure on the incidence of sister chromatid exchange in peripheral blood lymphocytes (Dalpra et al., 1983; Grandjean et al., 1983). A study by Dalpra et al. (1983) involved an investigation of the incidence of sister chromatid exchanges in 19 children who lived in a widely contaminated area and who showed increased lead absorption. Blood lead levels as well as erythrocyte ALA-D activity and zinc protoporphyrin in blood were measured in the exposed group as well as in the 12 controls. The exposed group had blood lead levels ranging from 29.3 to 62.7 µg/dl and ALA-D activity ranging from 8 to 32 erythrocytes. In contrast, the control group had blood lead values ranging from 10 to 21 µg/dl and ALA-D values ranging from 36 to 78/ml erythrocytes. Similarly, zinc protoporphyrin ranged from 65 to 341 µg/dl in the mU/ml exposed group and from 9 to 38 µg/dl in the controls. Thus, the population studied was well defined with regard to their exposure to lead. Based upon the measured parameters, the lead exposure was not severe. The results of an examination of sister chromatid exchange frequencies indicated no significant differences between the control group and the lead-exposed group. This appears to be a well-conducted study with excellent documentation of lead exposure in the population. The study examined the same exposed children that were observed by Forni et al. (1981) in their study of chromosomal aberrations. Forni et al. (1981), however, found an increased level of chromosomal aberration after 48- or 72-hr culture times (*vide supra*), suggesting that chromosomal aberrations may appear in the absence of detectable sister chromatid exchange (Dalpra et al., 1983). These findings emphasize the importance of utilizing a battery of test systems with different endpoints to accurately comprehend the true genotoxic potential of an agent.

Another recent well-conducted study, by Grandjean et al. (1983), examined the incidence of sister chromatid exchange in adult lead-exposed men. There was a significant correlation ($p < 0.001$) between the observed zinc protoporphyrin levels and the incidence of sister chromatid exchange in the lead-exposed group. However, there was a poor correlation between blood lead levels and sister chromatid exchange, which suggested that zinc protoporphyrin levels were a better indicator of lead exposure than blood lead levels in this study. Interestingly, during a 4-week cessation of lead exposure, the elevated incidence of sister chromatid exchange diminished, together with the zinc protoporphyrin levels and blood lead levels in perfused blood lymphocytes. The persistence of the sister chromatid exchange depends to a large extent upon the proliferation and half-life of the lymphocyte. In workers newly exposed to lead for four months there were clear increases in lead exposure parameters in the absence of any increase in the sister chromatid exchange frequency. Collectively, this study demonstrates for the first time a positive correlation between lead exposure and sister chromatid exchange. Further, it indicates that the increased sister chromatid exchange is not rapid in its induction, since it was only observed in lymphocytes after chronic exposure. Additionally, in lymphocytes the increased sister chromatid exchange was reversed when the lead exposure was decreased. It should be noted, however, that these effects occurred in only one cell type and the incidence of sister chromatid exchange may be uniquely different for every cell type *in vivo*. Nevertheless, these results support the potential injurious role lead may have on chromosomal structure and function.

The ability of agents such as lead to cause abnormal rearrangements in the structure of DNA, as revealed by the appearance of chromosomal aberrations and sister chromatid exchanges, has become an important focus in carcinogenesis research. Current theories suggest that cancer may result from an abnormal expression of oncogenes (genes that code for protein products associated with virally induced cancers). Numerous oncogenes are found in normal human DNA, but the genes are regulated such that they are not expressed in a carcinogenic fashion. Rearrangement of these DNA sequences within the genome is associated with oncogenesis, although the activation of oncogenes has not been demonstrated to be the cause of tumor induction. Evidence has been presented suggesting that chromosomal aberrations such as translocations are associated with certain forms of cancer and with the movement of oncogenes in regions of the DNA favoring their expression in cancer cells (Shen-Ong et al., 1982). By inducing aberrations in chromosomal structure, lead may enhance the probability of an oncogenic event.

12.7.3.3 Effects on Bacterial and Mammalian Mutagenesis Systems. Bacterial and mammalian mutagenesis test systems examine the ability of chemical agents to induce changes in DNA sequences of a specific gene product that is monitored by selection procedures. They measure the potential of a chemical agent to produce a change in DNA, but this change is not likely to be the same alteration in gene expression that occurs during oncogenesis. However, if an agent affects the expression of a particular gene product that is being monitored, then it could possibly affect other sequences that may result in cancer. Since many carcinogens are also mutagens, it is useful to employ such systems to evaluate genotoxic effects of lead.

Use of bacterial systems for assaying metal genotoxicity must await further development of bacterial strains that are appropriately responsive to known mutagenic metals (Rosenkranz and Poirier, 1979; Simmon, 1979; Simmon et al., 1979; Nishioka, 1975; Nestmann et al., 1979). Mammalian cell mutagenic systems that screen for specific alterations in a defined gene mutation have not been useful in detecting mutagenic activity with known carcinogenic metals (Heck and Costa, 1982b). In plants, however, chromosomal aberrations in root tips (Mukherji and Maitra, 1976) and other mutagenic activity, such as chlorophyll mutations (Reddy and Vaidyanath, 1978) and reproductive organ mutations (Lower et al., 1983), have been demonstrated with lead.

12.7.3.4 Effects on Parameters of DNA Structure and Function. There are a number of very sensitive techniques for examining the effect of metals on DNA structure and function in intact cells. Although these techniques have not been extensively utilized with respect to metal compounds, future research will probably be devoted to this area. Considerable work has been done to understand the effects of metals on enzymes involved in DNA replication.

Sirover and Loeb (1976) examined effects of lead and other metal compounds upon the fidelity of replication of DNA by a viral DNA polymerase. High concentrations of metal ions (in some cases in the millimolar range) were required to decrease the fidelity of replication, but there was a good correlation between metal ions that are carcinogenic or mutagenic and their activity in decreasing the fidelity of DNA replication. This assay system measures the ability of a metal ion to incorporate incorrect (non-homologous) bases using a defined polynucleotide template. In an intact cell, this would cause the induction of a mutation if the insertion of an incorrect base is phenotypically expressed. Since the interaction of metal ions with cellular macromolecules is relatively unstable, misincorporation of a base during semi-conservative DNA replication or during DNA repair synthesis following the induction of a DNA lesion with a metal could alter the base sequence of the DNA in an intact cell. Lead at 4 mM was among the metals listed as mutagenic or carcinogenic that caused a decrease in the fidelity of replication (Sirover and Loeb, 1976). Other metals active in decreasing fidelity included: Ag^+, Be^{2+}, Cd^{2+}, Co^{2+}, Cr^{2+}, Cr^{3+}, Cu^{2+}, Mn^{2+}, and Ni^{2+}. Metals that decreased fidelity are metals also implicated as carcinogenic or mutagenic (Sirover and Loeb, 1976).

In a similar study, Hoffman and Niyogi (1977) demonstrated that lead chloride was the most potent of 10 metals tested in inhibiting RNA synthesis (i.e., $Pb^{2+} > Cd^{2+} > Co^{2+} > Mn^{2+} > Li^+ > Na^+ > K^+$) for both types of templates tested, i.e., calf thymus DNA and T_4 phage DNA. These results were explained in terms of the binding of these metal ions more to the bases than to the phosphate groups of the DNA (i.e., $Pb^{2+} > Cd^{2+} > Zn^{2+} > Mn^{2+} > Mg^{2+} > Li^+ = Na^+ = K^+$). Additionally, metal compounds, such as lead chloride, with carcinogenic or mutagenic activity were found to stimulate mRNA chain initiation at 0.1 mM concentrations.

These well-conducted mechanistic studies provide evidence that lead can affect a molecular process associated with normal regulation of gene expression. Although far removed from the intact cell situation, these effects suggest that lead may be genotoxic. In a related study, lead sulfate along with numerous other toxic and carcinogenic metals was shown to cause an S-phase specific cell cycle block (Costa et al., 1982). A significant effect of lead was observed at 20 μM. These results indicate that this metal will interfere with the normal synthesis and replication of DNA. A recent study has examined the ability of lead acetate to induce strand breaks and DNA repair synthesis in cultured mammalian cells (Robinson et al., 1984). Lead acetate was slightly more potent than $NiCl_2$ in inducing true DNA single strand breaks, based upon neutral nucleoid gradient analysis, but was considerably less potent than $CaCrO_4$ or $HgCl_2$ (Robinson et al., 1984). Lead acetate also caused induction of DNA repair synthesis based upon analysis with CsCl equilibrium density gradient sedimentation. DNA repair synthesis was elevated about 10-fold above the control level at 200 μM lead acetate exposure for 1 hr (Robinson et al., 1984). These results further support the concept that lead can have effects upon the DNA.

12.7.4 Lead as an Initiator and Promoter of Carcinogenesis

An agent may act as a carcinogen in two distinct ways: (1) as an initiator; or (2) as a promoter (Weisburger and Williams, 1980). By definition, an initiator must be able to interact with DNA to produce a genetic alteration, whereas a promoter acts in a way that allows the expression of an altered genetic change responsible for cancer. Since lead is capable of transforming cells directly in culture and affecting DNA-to-DNA and DNA-to-RNA transcription, it may have some initiating activity. Its ability to induce chromosomal aberrations is also indicative of initiating activity. The ability of lead to induce proliferation in the kidney as indicated by increased DNA, RNA, and protein synthesis suggests that it may also have promoting activity in cancer target tissues (Choie and Richter, 1974a,b). Its similarity to Ca^{2+} suggests that it may alter regulation of this cation in processes (e.g., cell growth) related to promotion (see Section 12.3.5). A recent study, demonstrating that subsequent administration of basic lead acetate greatly enhances the development of renal tubular cell tumors in rats previously treated with n-ethyl-n-hydroxyethylnitrosamine, indicates a promotional role of this agent as well (Hiasa et al., 1983). Thus, evidence is accumulating to suggest that lead and its compounds are complete carcinogens possessing both initiating and promoting activity.

12.7.5 Summary and Conclusions

It is evident from studies reviewed above that, at relatively high concentrations, lead displays some carcinogenic activity in experimental animals such as the rat. Lead may act either as an initiator or promoter of carcinogenic activity, because it has genotoxic properties related to cancer initiation, as well as cellular effects related to the promotion or expression of cancer. The presence of intranuclear lead inclusion bodies in the kidney may pertain to lead's carcinogenic effects, since both the formation of these bodies and the induction of tumors occur at relatively high doses of lead. Evidence exists for the presence of these inclusion bodies in kidneys from experimental animals treated with lead and also in one well documented human case report of renal cancer associated with excessive lead exposure. The interaction of lead with key non-histone chromosomal proteins in the nucleus to form the inclusion bodies or the presence of inclusion bodies in the nucleus may alter genetic function, thus leading to cell transformation. Obviously, elucidating the mechanism of lead carcinogenesis requires further research efforts and only theories can be formulated regarding its oncogenic action at present.

It is hard to draw clear conclusions concerning what role lead may play in the induction of human neoplasia. Epidemiological studies of lead-exposed workers provide no definitive findings. However, statistically significant elevations in respiratory tract and digestive system cancer in workers exposed to lead and other agents warrant concern. Also, since lead acetate can produce renal tumors in some experimental animals, it may be prudent to assume that lead compounds may be carcinogenic in humans, as was concluded by the International Agency for Research on Cancer (1980). However, this statement is qualified by noting that lead has been observed to increase tumorigenesis rates in animals only at relatively high concentrations, and therefore it does not appear to be a potent carcinogen. A recent epidemiological study and two case reports suggest the possible association of lead exposure with the induction of kidney tumors in humans; however, several other epidemiological studies have not thus far demonstrated a significant excess of kidney tumors in lead workers. *In vitro* studies further support the genotoxic and carcinogenic role of lead, but also indicate that lead is not potent in these systems either.

12.8 EFFECTS OF LEAD ON THE IMMUNE SYSTEM

12.8.1 Development and Organization of the Immune System

Component cells of the immune system arise from a pool of pluripotent stem cells in the yolk sack and liver of the developing fetus and in the bone marrow and spleen of the adult. Stem cell differentiation and maturation follows one of several lines to produce lymphocytes, macrophages, and polymorphonuclear leukocytes. These cells have important roles in immunological function and host defense.

The predominant lymphocyte class develops in the thymus, which is derived from the third and fourth pharyngeal pouches at 9 weeks of gestation in man (day 9 in mice). In the thymus microenvironment, they acquire characteristics of thymus-derived lymphocytes (T-cells), then migrate to peripheral thymic-dependent areas of the spleen and lymph nodes. T-cells are easily distinguished from other lymphocytes by genetically defined cell surface markers that allow them to be further subdivided into immunoregulatory helper and suppressor T-cells. T-cells also participate directly as cytolytic effector cells against virally infected host cells, malignant cells, and foreign tissues, as well as in delayed-type hypersensitivity (DTH) reactions where they elaborate lymphokines that modulate the inflammatory response. T-cells are long-lived lymphocytes and are not readily replaced. Thus, any loss or injury to T-cells may be detrimental to the host and may result in increased susceptibility to viral, fungal, bacterial, or parasitic diseases. Individuals with acquired immune deficiency syndrome (AIDS) are examples of individuals with T-cell dysfunction. There is ample evidence that depletion by environmental agents of thymocytes or stem cell progenitors during lymphoid organogenesis can produce permanent immunosuppression.

The second major lymphocyte class differentiates from a lymphoid stem-cell in a yet undefined site in man, which would correspond functionally to the Bursa of Fabricius in avian species. In man, B-lymphocyte maturation and differentiation probably occur embryologically in gut-associated lymphoid tissue (GALT) and fetal liver, as well as adult spleen and bone marrow. This is followed by the peripheral population of thymic-independent areas of spleen and lymph nodes. Bone marrow-derived lymphocytes (B-cells), which mature independently of the thymus, possess specific immunoglobulin receptors on their surfaces. The presence of cell surface immunoglobulin (sIg) at high density is the major characteristic separating B-cells from T-cells. Following interaction with antigens and subsequent activation, B-lymphocytes proliferate and differentiate into antibody-producing plasma cells. In contrast to the long-lived T-cell, B-cells are rapidly replaced by newly differentiating stem cells. Therefore, lesions in the B-cell compartment may be less serious than those in the T-cell compartment since they are more easily reversed. Insult to B-cells at the stem cell or terminal maturation stage can result in suppression of specific immunoglobulin and enhanced susceptibility to infectious agents whose pathogenesis is limited by antibodies.

Pluripotent stem cells also give rise to lymphocytes whose lineages are still unclear. Some possess natural cytolytic activity for tumor cells (natural killer cell activity), while others, devoid of T- and B-cell surface markers (null cells), participate in antibody-dependent cell-mediated cytotoxicity (ADCC). The pluripotent stem cell pool also contains precursors of monocyte-macrophages and polymorphonuclear leukocytes (PMN). The macrophage has a major role in presentation and processing of certain antigens, in cytolysis of tumor target cells, and in phagocytosis and lysis of persistent intracellular infectious agents. Also, it actively phagocytizes and kills invading organisms. Defects in differentiation or function of PMNs or macrophages predispose the host to infections by bacteria and other agents.

This introduction should make it evident that the effects of an element such as lead on the immune system may be expressed in complex or subtle ways (see also Section 12.3.5.3.5). In some cases, lead might produce a lesion of the immune system not resulting in markedly adverse health effects, especially if the lesion did not occur at an early stem cell stage or during a critical point in lymphoid organogenesis. On the other hand, some lead-induced immune system effects might adversely affect health through increasing susceptibility to infectious agents or neoplastically transformed cells if, for example, they were to impair cytocidal or bactericidal function.

12.8.2 Host Resistance

One way of ascertaining if a chemical affects the immune response of an animal is to challenge an exposed animal with a pathogen such as an infectious agent or oncogen. This provides a general approach to determine if the chemical interferes with host immune defense mechanisms. Host defense is a composite of innate immunity, part of which is phagocyte activities, and acquired immunity, which includes B- and T-lymphocyte and enhanced phagocyte reactivities. Analysis of host resistance constitutes a holistic approach. However, dependent on the choice of the pathogen, host resistance can be evaluated somewhat more selectively. Assessment of host resistance to extracellular microbes such as *Staphylococci*, *Salmonella typhimurium*, *Escherichia coli*, or *Streptococcus pneumoniae* and to intracellular organisms such as *Listeria monocytogenes* or *Candida albicans* primarily measures intact humoral immunity and cell-mediated immunity, respectively. Immune defense to extracellular organisms requires T-lymphocyte, B-lymphocyte, and macrophage interactions for the production of specific antibodies to activate the complement cascade and to aid phagocytosis. Antibodies can also directly neutralize some bacteria and viruses. Resistance to intracellular organisms requires T-lymphocyte and macrophage interactions for T-lymphocyte production of lymphokines, which further enhance immune mechanisms including macrophage bactericidal activities. An additional T-lymphocyte subset, the cytolytic T-cell, is involved in resistance to tumors; immune defenses against tumors are also aided by NK- and K-lymphocytes and macrophages.

12.8.2.1 Infectivity Models. Numerous studies designed to assess the influence of lead on host resistance to infectious agents consistently have shown that lead impairs host resistance, regardless of whether the defense mechanisms are predominantly dependent on humoral- or cell-mediated immunity (Table 12-23).

TABLE 12-23. EFFECT OF LEAD ON HOST RESISTANCE TO INFECTIOUS AGENTS

Species	Infectious agent	Lead dose	Lead exposure	Mortality*	Reference
Mouse	*S. typhimurium*	200 ppm	i.p.; 30 days	54% (13%)	Hemphill et al. (1971)
Rat	*E. coli*	2 mg/100 g body weight	i.v.; 1 day	96% (0%)	Cook et al. (1975)
Rat	*S. epidermidis*	2 mg/100 g body weight	i.v.; 1 day	80% (0%)	Cook et al. (1975)
Mouse	*L. monocytogenes*	80 ppm	orally; 4 wk	100% (0%)	Lawrence (1981a)
Mouse	EMC† virus	2000 ppm	orally; 2 wk	100% (19%)	Gainer (1977b)
Mouse	EMC virus	13 ppm	orally; 10 wk	80% (50%)	Exon et al. (1979)
Mouse	Langat virus	50 mg/kg body weight	orally; 2 wk	68% (0%)	Thind and Khan (1978)

*The percent mortality is reported for the lowest dose of lead in the study that significantly altered host resistance. The percent mortality in parentheses is that of the non-lead-treated, infected control group.

†EMC = encephalomyocarditis virus.

Mice (Swiss Webster) injected i.p. for 30 days with 100 or 250 µg (per 0.5 ml) of lead nitrate and inoculated with *Salmonella typhimurium* had higher mortality (54 and 100 percent, respectively) than non-lead-injected mice (13 percent) (Hemphill et al., 1971). These concentrations of lead, by themselves, did not produce any apparent toxicity. Similar results were observed in rats acutely exposed to lead (one i.v. dose of 2 mg/100 g body weight) and challenged with *Escherichia coli* (Cook et al., 1975). In these two studies, lead could have interfered with the clearance of endotoxin from the *S. typhimurium* or *E. coli*, and the animals may have died from endotoxin shock, and not septicemia, due to the lack of bacteriostatic or bactericidal activities. However, the study by Cook et al. (1975) also included a non-endotoxin-producing gram-positive bacterium, *Staphylococcus epidermidis*, and lead still impaired host resistance. In another study, lead effects on host resistance to the intracellular parasite *Listeria monocytogenes* were monitored (Lawrence, 1981a). CBA/J mice orally exposed to 16, 80, 400, and 2000 ppm lead for four weeks were assayed for viable *Listeria* after 48 and 72 hours, and for mortality after 10 days. Only 2000 ppm lead caused significant inhibition of early bactericidal activity (48-72 hr), but 80-2000 ppm lead produced 100 percent mortality, compared with 0 percent mortality in the 0-16 ppm lead groups. Other reports have suggested that host resistance is impaired by lead exposure of rodents. Salaki et al. (1975) indicated that lead lowered resistance of mice to Staphylococcus aureus, Listeria, and Candida, and observed higher incidence of inflammation of the salivary glands in lead-exposed rats (Grant et al., 1980) may be due, in part, to lead-induced increased susceptibility to infections.

Inhalation of lead has also been reported to lower host resistance to bacteria. Schlipköter and Frieler (1979) exposed NMRI mice to an aerosol of 13-14 $\mu g/m^3$ lead chloride and clearance of Serratia marcesens in the lungs was reduced significantly. Microparticles of lead in lungs of mice were also shown to lower resistance to Pasteurella multocida, in that 6 μg of lead increased the percentage of mortality by 27 percent (Bouley et al., 1977).

Lead has also been shown to increase host susceptibility to viral infections. CD-1 mice, administered 2,000 and 10,000 ppm lead in drinking water for two weeks and subsequently inoculated with encephalomyocarditis (EMC) virus, had a significant increase in mortality (100 percent at 2,000 ppm; 65 percent at 10,000 ppm) compared with control EMC virus-infected mice (13 percent) (Gainer, 1977b). In another study (Exon et al., 1979), Swiss Webster mice were exposed to 13, 130, 1300, or 2600 ppm lead for 10 weeks in their drinking water and were infected with EMC virus. Although as low as 13 ppm lead caused a significant increase in mortality (80 percent) in comparison with the non-lead-treated EMC virus-infected mice (50 percent), there were no dose-response effects, in that 2600 ppm lead resulted in only 64 percent mortality. The lack of a dose-response relationship in the two studies with EMC virus (Gainer, 1977b; Exon et al., 1979) suggests that the higher doses of lead may directly inhibit EMC infectivity as well as host defense mechanisms. Additional studies have confirmed that lead inhibits host resistance to viruses. Mice treated orally with lead nitrate (10-50 mg/kg/ day) for two weeks had suppressed antibody titers to Langat virus (Type B arbovirus) and increased titers of the virus itself (Thind and Singh, 1977), and the lead-inoculated, infected mice had higher mortalities (25 percent at 10 mg/kg; 68 percent at 50 mg/kg) than the non-lead-infected mice (0 percent) (Thind and Khan, 1978).

The effects of lead on bacterial and viral infections in humans have never been studied adequately; there is only suggestive evidence that human host resistance may be lowered by lead. Children with persistently high blood lead levels who were infected with Shigella enteritis had prolonged diarrhea (Sachs, 1978). In addition, lead workers with blood lead levels of 22-89 μg/dl have been reported to have more colds and influenza infections per year (Ewers et al., 1982). This study also indicated that secretory IgA levels were suppressed significantly in lead workers with a median blood lead level of 55 μg/dl. Secretory IgA is a major factor in immune defense against respiratory as well as gastrointestinal infections.

Hicks (1972) points out that there is need for systematic epidemiological studies on the effects of elevated lead levels on the incidence of infectious diseases in humans. The current paucity of information precludes formulation of any clear dose-response relationship for humans. Epidemiological investigations may help to determine if lead alters the immune system of man and consequently increases susceptibility to infectious agents and neoplasia.

12.8.2.2 Tumor Models and Neoplasia. The carcinogenicity of lead has been studied both as a direct toxic effect of lead (see Section 12.7) and as a means of better understanding the effects of lead on the body's defense mechanisms. Studies by Gainer (1973, 1974) demonstrated that exposure of CD-1 mice to lead acetate potentiated the oncogenicity of a challenge with Rauscher leukemia virus (RLV), resulting in enhanced splenomegaly and higher virus titers in the spleen presumably through an immunosuppressive mechanism. Recent studies by Kerkvliet and Baecher-Steppan (1982) revealed that chronic exposure of C57BL/6 mice to lead acetate in drinking water at 130-1300 ppm enhanced the growth of primary tumors induced by Moloney sarcoma virus (MSV). Regression of MSV-induced tumors was not prevented by lead exposure, and lead-treated animals resisted late sarcoma development following primary tumor resistance. Depressed resistance to transplantable MSV tumors was associated with a reduced number of macrophages, which also exhibited reduced phagocytic activity.

In addition to enhancing the transplantability of tumors or the oncogenicity of leukemia viruses, lead has also been shown to facilitate the development of chemically induced tumors. Kobayashi and Okamoto (1974) found that intratracheal dosing of benzo(a)pyrene (BaP) combined with lead oxide resulted in an increased frequency of lung adenomas and adenocarcinomas over hamsters exposed to BaP alone. Similarly, exposure to lead acetate enhanced the formation of N(4'-fluoro-4-biphenyl) acetamide-induced renal carcinomas from 70 to 100 percent and reduced the latency to tumor appearance (Hinton et al., 1980). Recently, Koller et al. (1985) found that exposure to lead (2600 ppm in drinking water) for 18 months increased the frequency of tumors, predominantly renal carcinomas, in rats. Similarly, Schrauzer et al. (1981) found that adding lead at 5 ppm to drinking water of C3H/St mice infected with Bittner milk factor diminished the uptake of selenium and reduced its anticarcinogenic effects, causing mammary tumors to appear at the same high incidence as in selenium-unsupplemented controls. Lead likewise significantly accelerated tumor growth and shortened survival in this model.

The above studies on host susceptibility to various pathogens, including infectious agents and tumors, indicate that lead could be detrimental to health by methods other than direct toxicity. In order to understand the mechanisms by which lead suppresses host resistance maintained by phagocytes, humoral immunity, and/or cell-mediated immunity, the immune system must be dissected into its functional components and the effects of lead on each, separately and combined, must be examined in order that the mechanism(s) of the immunomodulatory potential of lead can be understood.

12.8.3 Humoral Immunity

12.8.3.1 Antibody Titers. A low antibody titer in animals exposed to lead could explain the increased susceptibility of animals to extracellular bacteria and some viruses (see Table 12-24), as well as to endotoxins (Selye et al., 1966; Filkins, 1970; Cook et al., 1974; Schumer and Erve, 1973; Rippe and Berry, 1973; Truscott, 1970). Specific antibodies can directly neutralize pathogens, activate complement components to induce lysis, or directly or indirectly enhance phagocytosis via Fc or C3 receptors, respectively. Studies in animals and humans have assayed the effects of lead on serum immunoglobulin levels, specific antibody levels, and complement levels. Analysis of serum immunoglobulin levels is not a good measure of specific immune reactivity, but it would provide evidence for an effect on B-lymphocyte development.

TABLE 12-24. EFFECT OF LEAD ON ANTIBODY TITERS

Species	Antigen	Lead dose and exposure	Effect on antibody titer	Reference
Rabbit	Pseudorabies virus	2,500 ppm; 10 wk	Decrease	Koller (1973)
Rat	*S. typhimurium*	5,000-20,000 ppm; 3 wk	Decrease	Stanković and Jugo (1976)
Rat	Bovine serum albumin	10-1,000 ppm; 10 wk	Decrease	Koller et al. (1983)
Mouse	Sheep erythrocytes	0.5-10 ppm[a]; 3 wk	Decrease	Blakley et al. (1980)

[a] Lead was administered as tetraethyl lead; other studies used inorganic forms.

Lead had little effect on the serum immunoglobulin levels in rabbits (Fonzi et al., 1967a), children with blood lead levels of 40 μg/dl (Reigart and Graber, 1976), or lead workers with 22-89 μg/dl (Ewers et al., 1982). On the other hand, most studies have shown that lead significantly impairs antibody production. Acute oral lead exposure (50,000 ppm/kg) produced a decreased titer of anti-typhus antibodies in rabbits immunized with typhus vaccine (Fonzi et al., 1967b). In New Zealand white rabbits challenged with pseudorabies virus, lead (oral exposure to 2500 ppm for 70 days) caused a 9-fold decrease in antibody titer to the virus (Koller, 1973). However, lead has not always been shown to reduce titers to virus. Vengris and Mare (1974) did not observe depressed antibody titers to Newcastle disease virus in lead-treated chickens, but their lead treatment was only for 35 days prior to infection. Lead-poisoned children also had normal anti-toxoid titers after booster immunizations with tetanus toxoid (Reigart and Graber, 1976). In another study, Wistar rat dams were exposed to 5,000, 10,000, or 20,000 ppm lead for 20 days following parturition (Stanković and Jugo, 1976). The progeny were weaned at 21 days of age and given standard laboratory chow for an additional month. At that time, they were injected with Salmonella typhimurium, and serum antibody titers were assessed. Each dosage of lead resulted in significantly reduced antibody titers. More recently, rats (Sprague-Dawley) given 10 ppm lead acetate orally for 10 weeks had a significant suppression in antibody titers when challenged with bovine serum albumin (BSA) and compared with BSA-immunized non-lead-exposed rats (Koller et al., 1983). Development of a highly sensitive, quantitative, enzyme-linked immunosorbent assay (ELISA) contributed to detecting the immunosuppressive activity of lead at this dosage.

Tetraethyl lead also has been responsible for reduced antibody titers in Swiss-cross mice (Blakley et al., 1980). The mice were exposed orally to 0.5, 1.0, and 2.0 ppm tetraethyl lead for 3 weeks. A significant reduction in hemagglutination titers to sheep red blood cells (SRBC) occurred at all levels of exposure.

12.8.3.2 Enumeration of Antibody Producing Cells (Plaque-Forming Cells). From the above results, it appears that lead inhibits antibody production. To evaluate this possible effect at the cellular level, the influence of lead on the number of antibody-producing cells after primary or secondary immunization can be assessed. In primary humoral immune responses (mostly direct), IgM plaque-forming cells (PFC) are measured, whereas in secondary or anamnestic responses (mostly indirect), IgG PFC are counted. The primary immune response represents an individual's first contact with a particular antigen. The secondary immune response represents re-exposure to the same antigen weeks, months, or even years after the primary antibody response has subsided. The secondary immune response is attributed to persistence, after initial contact with the antigen, of a substantial number of antigen-sensitive memory cells. Impairment of the memory response, therefore, results in serious impairment of humoral immunity in the host.

Table 12-25 summarizes the effects of lead on IgM or IgG PFC development. Mice exposed orally to tetraethyl lead (0.5, 1, or 2 ppm) for three weeks exhibited a significant reduction in the development of IgM and IgG PFC (Blakley et al., 1980). Mice (Swiss Webster) exposed orally to 13, 137, or 1375 ppm inorganic lead for eight weeks had reduced numbers of IgM PFC in each lead-exposed group (Koller and Kovacic, 1974). Even the lowest lead group (13 ppm) had a decrease. The secondary response (IgG PFC, induced by a second exposure to antigen SRBC seven days after the primary immunization) was inhibited to a greater extent than the primary response. This study indicated that chronic exposure to lead produced a significant decrease in the development of IgM PFC and IgG PFC. When Swiss Webster mice were exposed to 13, 130, and 1300 ppm lead for 10 weeks and hyperimmunized by SRBC injections at week 1, 2, and 9, the

TABLE 12-25. EFFECT OF LEAD ON THE DEVELOPMENT OF ANTIBODY-PRODUCING CELLS

Species	Antigen*	Lead dose and exposure	Effect†	Reference
Mouse	SRBC (*in vivo*)	13-1370 ppm; 8 wk	IgM PFC (D) IgG PFC (D)	Koller and Kovacic (1974)
Mouse	SRBC (*in vivo*)	0.5-2 ppm tetraethyl lead; 3 wk	IgM PFC (D) IgG PFC (D)	Blakley et al. (1980)
Mouse	SRBC (*in vivo*)	13-1370 ppm; 10 wk	IgG PFC (D)	Koller and Roan (1980a)
Mouse	SRBC (*in vivo*)	4 mg (i.p. or orally)	IgM PFC (I) IgG PFC (D)	Koller et al. (1976)
Mouse	SRBC (*in vivo*) SRBC (*in vitro* + 2-ME)	16-2000 ppm; 1-10 wk 16-80 ppm; 4 wk 2000 ppm; 4 wk	IgM PFC (N) IgM PFC (I) IgM PFC (D)	Lawrence (1981a)
Rat	SRBC (*in vivo*)	25-50 ppm; pre/postnatal	IgM PFC (D)	Luster et al. (1978)
Mouse	SRBC (*in vitro*) SRBC (*in vitro* + 2-ME)	50-1000 ppm; 3 wk 50-1000 ppm; 3 wk	IgM PFC (D) IgM PFC (N or I)	Blakley and Archer (1981)
Mouse	SRBC (*in vitro* + 2-ME)	2-20 ppm (*in vitro*)	IgM PFC (I)	Lawrence (1981b,c)

*The antigenic challenge with sheep red blood cells (SRBC) was *in vivo* or *in vitro* after *in vivo* exposure to lead unless otherwise stated. The *in vitro* assays were performed in the presence or absence of 2-mercaptoethanol (2-ME).

†IgM/G PFC = immunoglobulin M/G plaque-forming cells; D = decreased response; N = unaltered response; I = increased response.

memory response as assessed by the enumeration of IgG PFC was significantly inhibited at 1300 ppm (Koller and Roan, 1980a). This suggests that the temporal relationships between lead exposure and antigenic challenge may be critical. Other studies support this interpretation. Female Sprague-Dawley rats with pre- and post-natal exposure to lead (25 or 50 ppm) had a significant reduction in IgM PFC (Luster et al., 1978). In contrast, CBA/J mice exposed orally to 16-2000 ppm lead for 1-10 weeks did not have altered IgM PFC responses to SRBC (Lawrence, 1981a). Furthermore, when Swiss Webster mice were exposed to an acute lead dose (4 mg lead orally or i.p.), the number of IgG PFC was suppressed, but the number of IgM PFC was enhanced (Koller et al., 1976).

The influence of lead on the development of PFC in mice was assessed further by *in vivo* exposure to lead, removal of spleen cells, and *in vitro* analysis of PFC development. Initially it appeared that low doses of lead (16 and 80 ppm) enhanced development, and only a high dose (2000 ppm) inhibited the development of IgM PFC (Lawrence, 1981a). However, a later study by Blakley and Archer (1981) indicated that 50-1000 ppm lead consistently inhibited IgM PFC. Through the analysis of mixed cultures of lead-exposed lymphocytes (nonadherent cells) and unexposed macrophages (adherent cells), and vice versa, as well as of *in vitro* responses to antigens that do not require macrophage help (i.e., lipopolysaccharide, LPS), their data indicated that the effects of lead may be at the level of the macrophage. This was substantiated by the fact that 2-mercaptoethanol (2-ME), a compound that can substitute for at least one macrophage activity, was able to reverse the inhibition by lead. This may explain why *in vivo* lead exposure (16 and 80 ppm) appeared to enhance the *in vitro* IgM PFC responses in the study by Lawrence (1981a), because 2-ME was present in the *in vitro* assay system. Furthermore, *in vitro* exposure to lead (2 or 20 ppm) in spleen cell cultures with 2-ME enhanced the development of IgM PFC (Lawrence, 1981b,c).

These experiments indicate that lead modulates the development of antibody-producing cells as well as serum antibody titers, which supports the notion that lead can suppress humoral immunity. However, it should be noted that the dose and route of exposure of both lead and antigen may influence the modulatory effects of lead. The adverse effects of lead on humoral immunity may be due more to lead's interference with macrophage antigen processing and/or antigen presentation to lymphocytes than to direct effects on B-lymphocytes. These mechanisms require further investigation.

12.8.4 Cell-Mediated Immunity

12.8.4.1 Delayed-Type Hypersensitivity. T-lymphocytes (T-helper and T-suppressor cells) are regulators of humoral and cell-mediated immunity as well as effectors of two aspects of cell-mediated immunity. T-cells responsive to delayed-type hypersensitivity (DTH) produce lymphokines that induce mononuclear infiltrates and activate macrophages, which are aspects of chronic inflammatory responses. In addition, another subset of T-cells, cytolytic T-cells, cause direct lysis of target cells (tumors or antigenically modified autologous cells) when in contact with the target. To date, the effects of lead on cytolytic T-cell reactivity have not been measured, but the influence of lead on inducer T-cells has been studied (Table 12-26). Groups of mice injected i.p. daily for 30 days with 13.7-137 ppm lead were subsequently sensitized i.v. with SRBC. The DTH reaction was suppressed in these animals in a dose-related fashion (Müller et al., 1977). The secondary DTH response was inhibited in a similar fashion. In another study (Faith et al., 1979), the effects of chronic low-level pre- and postnatal lead exposure on cellular immune functions in Sprague-Dawley rats were assessed. Female rats

TABLE 12-26. EFFECT OF LEAD ON CELL-MEDIATED IMMUNITY

Species	Lead dose and exposure	Parameter*	Effect	Reference
Mouse	13.7-137 ppm; 4 wk	DTH	Decrease	Müller et al. (1977)
Rat	25-50 ppm; 8 wk	DTH	Decrease	Faith et al. (1979)
Mouse	13-1300 ppm; 10 wk	MLC	None	Koller and Roan (1980b)
Mouse	16-2000 ppm; 4 wk	MLC	Decrease	Lawrence (1981a)

*DTH = delayed-type hypersensitivity; MLC = mixed lymphocyte culture.

were exposed to 25 or 50 ppm lead acetate continuously for seven weeks before breeding and through gestation and lactation. The progeny were weaned at three weeks of age and continued on the respective lead exposure regimen of their mothers for an additional 14-24 days. Thymic weights and DTH responses were significantly decreased by both lead dosages. These results indicate that chronic low levels of lead suppress cell-mediated immune function.

The _in vitro_ correlate of the analysis of DTH responsive T-cells _in vivo_ is the analysis of mixed lymphocyte culture (MLC) responsive T-cells. When two populations of allogeneic lymphoid cells are cultured together, cellular interactions provoke blast cell transformation and proliferation of a portion of the cultured cells (Cerottini and Brunner, 1974; Bach et al., 1976). The response can be made one-way by irradiating one of the two allogeneic preparations, in which case the irradiated cells are the stimulators (allogeneic B-cells and macrophages) and the responders (T-cells) are assayed for their proliferation. The mixed lymphocyte reaction is an _in vitro_ assay of cell-mediated immunity analogous to _in vivo_ host versus graft reactions.

Mice (DBA/2J) fed 13, 130, or 1300 ppm lead for 10 weeks were evaluated for responsiveness in mixed lymphocyte cultures. The 130-ppm lead dose tended to stimulate the lymphocyte reaction, although no change was observed at the other dose levels (Koller and Roan, 1980b). In another study (Lawrence, 1981a), mice (CBA/J) were fed 16, 80, 400, or 2000 ppm lead for four weeks. The 16 and 80 ppm doses slightly stimulated, while the 2000 ppm dose suppressed, the mixed lymphocyte reaction. It is important to note that in these _in vitro_ MLC assays, 2-ME was present in the culture medium, and the 2-ME may have reversed the _in vivo_ effects of lead, as was observed for the _in vitro_ PFC responses (Blakley and Archer, 1981).

The data on the effects of lead on humoral and cell-mediated immunity indicate that _in vivo_ lead usually is immunosuppressive, but additional studies are necessary to fully understand the temporal and dose relationship of lead's immunomodulatory effects. The _in vitro_ analysis of immune cells exposed to lead *in vivo* suggest that the major cell type modified is the macrophage; the suppressive effects of lead may be readily reversed by thiol reagents possibly acting as chelators.

12.8.4.3 Interferon. Interferons (IF) are a family of low-molecular-weight proteins which exhibit antiviral activity in sensitive cells through processes requiring new cellular RNA and protein synthesis (Stewart, 1979). It has been speculated that the enhanced susceptibility of lead-treated mice to infectious virus challenge might be due to a decreased capacity of these animals to produce viral or immune interferons or to respond to them. Studies by Gainer (1974, 1977a) appeared to resolve this question and indicated that exposure of CD-1 mice to lead does not inhibit the antiviral action of viral IF *in vivo* or *in vitro*. In the later of the two studies, lead exposure inhibited the protective effects of the IF inducers Newcastle disease virus and poly I:poly C against encephalomyocarditis virus (EMC)-induced mortality. These data suggest that, although lead did not directly interfere with the antiviral activity of interferon, it might suppress viral IF production *in vivo*. Recently, Blakley et al. (1982) re-examined this issue and found that female BDF_1 mice exposed to lead·acetate in drinking water at concentrations ranging from 50 to 1000 µg/ml for three weeks produced amounts of IF similar to controls given a viral IF inducer, Tilorone. Similarly, the *in vitro* induction of immune IF by the T-cell mitogens--phytohemagglutinin, concanavalin A, and staphylococcal enterotoxin--in lymphocytes from lead-exposed mice were unaltered compared with controls (Blakley et al., 1982). Thus, lead exposure does not appear to significantly alter the lymphocyte's ability to produce immune interferon. Therefore, it must be assumed that increased viral susceptibility associated with chronic lead exposure in rodents is by mechanisms other than interference with production of or response to interferon.

12.8.5 Lymphocyte Activation by Mitogens

Mitogens are lectins that induce activation, blast-cell transformation, and proliferation in resting lymphocytes. Certain lectins bind specifically to (1) T-cells (i.e., phytohemagglutinin [PHA] and concanavalin A [Con A]), (2) B-cells (i.e., lipopolysaccharide [LPS] of gram-negative bacteria), or (3) both (i.e., pokeweed mitogen [PWM]). The resulting blastogenic response can be used to assess changes in cell division of T- and B-lymphocytes. The biological significance of the following studies is difficult to interpret since exposure to lead was either *in vivo* or *in vitro* at different doses and for different exposure periods.

12.8.5.1 In Vivo Exposure. Splenic lymphocytes from Swiss Webster mice exposed orally to 2000 ppm lead for 30 days had significantly depressed proliferative responses to PHA (Table 12-27) which were not observed after 15 days of exposure (Gaworski and Sharma, 1978). Suppression was likewise observed with PWM, a T- and B-cell mitogen. These observations with

TABLE 12-27. EFFECT OF LEAD EXPOSURE ON MITOGEN ACTIVATION OF LYMPHOCYTES

Species	Lead dose and exposure	Mitogen[a]	Effect	Reference
Mice	In vivo, 250 and 2000 ppm, 30 days	PHA (T-Cell)	Significantly depressed at 2000 ppm on day 30 only[b]	Gaworski and Sharma (1978)
		PWM (T and B-Cell)	Significantly depressed at 2000 ppm on both days 15 and 30[b]	
Mice	In vivo, 13, 130, and 1300 ppm, 10 weeks	Con A (T-Cell)	No effect	Koller et al. (1979)
		LPS (B-Cell)	No effect	
Rats	In vivo, pre/postnatal 25 and 50 ppm, 7 weeks	Con A	Significantly depressed at 25 and 50 ppm	Faith et al. (1979)
		PHA	Significantly depressed at 50 ppm only	
Mice	In vivo, 0.08-10 mM, 4 weeks	Con A, PHA	No effect	Lawrence (1981c)
		LPS	Depressed at 2 and 10 mM	
Mice	In vivo, 1300 ppm, 8 weeks	Con A, PHA	Significantly depressed	Neilan et al. (1980)
		LPS	No effect	
Mice	In vivo, 50, 200, and 1000 ppm, 3 weeks	Con A, PHA, SEA	Increased to all[c]	Blakley and Archer (1982)
		LPS	No effect	
Mice	In vitro, 10^{-4}-10^{-6} M for full culture period	Con A, PHA	Slightly increased at highest dose at day 2, no effect at day 3.5	Lawrence (1981a,b)
		LPS	Increased up to 245%[b]	
Mice	In vitro, 0.1, 0.5, 1.0 mM for full culture period	PHA	Increased at all doses by up to 453%[d]	Gaworski and Sharma (1978)
		PWM	Increased by approximately 250% at 0.1 and 0.5 mM only	
Mice	In vitro, 10^{-3}-10^{-7} M for 72 hours	LPS	Increased by up to 312%	Shenker et al. (1977); Gallagher et al. (1979)

[a] PHA = phytohemagglutinin; PWM = pokeweed mitogen; Con A = concanavalin A; LPS = lipopolysaccharide; SEA = staphylococcal enterotoxin A.

[b] Difficult to interpret since data were reported only as % of control response rather than CPM of thymidine incorporation.

[c] Untreated control values unusually low for T-cell response. Lead-treated had much higher response with highest dose showing cytotoxicity.

[d] Noted white precipitate thought to be lead carbonate in cultures.

T-cell mitogens were confirmed in Sprague-Dawley rats exposed orally to 25 or 50 ppm lead pre- and postnatally for seven weeks (Faith et al., 1979). Splenic T-cell responses to Con A and PHA were significantly diminished. A similar depression of Con A and PHA responses occurred in lymphocytes from C57Bl/6 mice exposed to 1300 ppm lead for 8 weeks (Neilan et al., 1980). Lead impaired blastogenic transformation of lymphocytes by both T-cell mitogens, although the B-cell proliferative response to LPS was not impaired.

In contrast to reports that lead exposure suppressed the blastogenic response of T-cells to mitogens, several laboratories have reported that lead exposure does not suppress T-cell proliferative responses (Koller et al., 1979; Lawrence, 1981c; Blakley and Archer, 1982). These differences are not easily reconciled since analysis of the lead dose employed and exposure period (Table 12-26) provides little insight into the observed differences in T-cell responses. In one case, a dose of 2000 ppm for 4 weeks produced a clear depression, while a lesser dose of 1300 ppm produced no effect at 10 weeks in another laboratory. These data are confusing and may reflect technical differences in performing the T-cell blastogenesis assay in different laboratories, a lack of careful attention to lectin response kinetics, or the influence of suppressor macrophages. Thus, no firm conclusion can be drawn regarding the ability of in vivo exposure to lead to impair the proliferative capacity of T-cells.

The blastogenic response of B-cells to LPS was unaffected in four different in vivo studies at lead exposure levels of 25-1300 ppm (Koller et al., 1979; Faith et al., 1979; Neilan et al., 1980; Blakley and Archer, 1982). Lawrence (1981c), however, reported that the LPS response was suppressed after 4 weeks exposure at 2 and 10 mM lead. The weight of the data suggests that the proliferative response of B-cells to LPS is probably not severely impaired by lead exposure.

12.8.5.2 In Vitro Exposure. The biological relevance of immunological studies in which lead was added in vitro to normal rodent splenocytes in the presence of a mitogen (Table 12-27) is questionable since differences probably reflect either a direct toxic or stimulatory effect by the metal. These models may, however, provide useful information regarding metabolic and functional responses in lymphocytes using lead as a probe.

In one study, lymphocytes were cultured in the presence of lead (10^{-4}, 10^{-5}, and 10^{-6} M). A slight but significant increase in lymphocyte transformation occurred on day 2 at the highest lead dosage when stimulated with Con A or PHA (Lawrence, 1981b). In a follow-up study where the kinetics of the lectin response were examined (Lawrence, 1981a), lead (10^{-4}, 10^{-5}, and 10^{-6} M) significantly suppressed the Con A- and PHA-induced proliferative responses of lymphocytes on day 2, but not on days 3-5. In yet another in vitro exposure study, lymphocytes cultured in the presence of 0.1, 0.5, or 1.0 mM lead had a significantly enhanced response to PHA (Gaworski and Sharma, 1978). It should be kept in mind when considering these *in vitro* exposure observations that lead has been demonstrated to be directly mitogenic to lymphocytes (Shenker et al., 1977). The data discussed here suggest that lead may also be slightly co-mitogenic with T-cell mitogens. Direct exposure of lymphocytes in culture to lead can also result in decreased lymphocyte viability (Gallagher et al., 1979). *In vitro* studies on the effect of lead on the B-cell blastogenic response to LPS indicated that lead is potently co-mitogenic with LPS and enhanced the proliferative response of B-cells by 245 percent (Lawrence 1981b,c) to 312 percent (Shenker et al., 1977; Gallagher et al., 1979).

12.8.6 Macrophage Function

The monocyte/macrophage is involved with phagocytosis, bactericidal activity, processing of complex antigens for initiation of antibody production, interferon production, endotoxin detoxification, and immunoregulation. Since some of these functions are altered in lead-treated rodents (Table 12-28), the monocyte/macrophage or comparable phagocytic cell in the liver has been suggested as a possible cellular target for lead (Trejo et al., 1972; Cook et al., 1974; Müller et al., 1977; Luster et al., 1978; Blakley and Archer, 1981).

Several laboratories have shown that a single i.v. injection of lead impaired the phagocytic ability of the reticuloendothelial system (RES) (Trejo et al., 1972; Cook et al., 1974; Filkins and Buchanan, 1973). Trejo et al. (1972) found that an i.v. injection of 5 mg lead impaired vascular clearance of colloidal carbon that resulted from an impaired phagocytic ability of liver Kupffer cells. Similarly, others have confirmed that lead injected i.v. depressed intravascular clearance of colloidal carbon (Filkins and Buchanan, 1973) as well as a radiolabeled lipid emulsion (Cook et al., 1974). Opposite effects on RES function have been seen when lead was given orally (Koller and Roan, 1977). Similarly, Schlick and Friedberg (1981) noted that a 10-day exposure to 10-1000 μg lead enhanced RES clearance and endotoxin hypersensitivity.

Lead has likewise been demonstrated to suppress macrophage-dependent immune responses (Blakley and Archer, 1981). Exposure of BDF_1 mice to lead (50 ppm) for three weeks in drinking water suppressed *in vitro* antibody PFC responses to the macrophage-dependent antigens, sheep red blood cells or dinitrophenyl-Ficoll, but not to the macrophage-independent antigen, *E. coli* lipopolysaccharide. The macrophage substitute, 2-mercaptoethanol, and macrophages from non-exposed mice restored lead-suppressed response. Castranova et al. (1980) found that cultured rat alveolar macrophages exposed to lead had depressed oxidative metabolism.

The effects of heavy metals on endotoxin hypersensitivity were first observed by Selye et al. (1966), who described a 100,000-fold increase in bacterial endotoxin sensitivity in rats given lead acetate. The increased sensitivity to endotoxin was postulated to be due to a blockade of the RES. Filkins (1970) subsequently demonstrated that endotoxin detoxification

TABLE 12-28. EFFECT OF LEAD ON MACROPHAGE AND RETICULOENDOTHELIAL SYSTEM FUNCTION

Species	Lead dose and mode	Parameter	Effect	Reference
Rat	2.25 μmol, single intravenous injection	Vascular clearance; lipid emulsion endotoxin sensitivity	Depressed Increased	Cook et al. (1974); Trejo et al. (1972)
Rat	5 mg, single intravenous injection	Vascular clearance; colloidal carbon endotoxin sensitivity	Depressed Increased	Trejo et al. (1972); Filkins and Buchanan (1973)
Mouse	13, 130, 1300 ppm oral, 10-12 weeks	Phagocytosis	Depressed	Kerkvliet and Baecher-Steppan (1982)
Guinea Pig	10^{-3}-10^{-6}M, *in vitro*	Macrophage migration	Depressed	Kiremidjian-Schumacher et al. (1981)
Rat	10^{-3}-10^{-6}M, *in vitro*	Macrophage oxygen metabolism	Depressed	Castranova et al. (1980)
Mouse	50-1000 ppm oral, 3 weeks	Plague-forming cell response to macrophage dependent antigens	Depressed	Blakley and Archer (1981)
Mouse	10-1000 μg, 10 days, intravenous injection	Vascular clearance	Enhanced at 10 days; no effect at >30 days	Schlick and Friedberg (1981)
		Endotoxin sensitivity	Increased	

is primarily a hepatic macrophage-mediated event that is profoundly impaired by lead exposure (Trejo and Di Luzio, 1971; Filkins and Buchanan, 1973). The several types of data described above suggest that macrophage dysfunction may be contributing to impairment of immune function, endotoxin detoxification, and host resistance following lead exposure.

12.8.7 Mechanisms of Lead Immunomodulation

The mechanism of toxic action of lead on cells is complex (see Section 12.2). Since lead has a high affinity for sulfhydryl groups, a likely subcellular alteration accounting for the immunomodulatory effects of lead on immune cells is its association with cellular thiols. Numerous studies have indicated that surface and intracellular thiols are involved in lymphocyte activation, growth, and differentiation. Furthermore, the study by Blakley and Archer (1981) suggests that lead may inhibit the macrophage's presentation of stimulatory products to the lymphocytes. This process may rely on cellular thiols since the inhibitory effects of lead can be overcome by an exogenous thiol reagent. Goyer and Rhyne (1973) have indicated that lead ions tend to accumulate on cell surfaces, thereby possibly affecting surface receptors and cell-to-cell communication. A study by Koller and Brauner (1977) indicated that lead does alter C3b binding to its cell surface receptor.

12.8.8 Summary

Lead renders animals highly susceptible to endotoxins and infectious agents. Host susceptibility and the humoral immune system appear to be particularly sensitive. As postulated in recent studies, the macrophage may be the primary immune target cell of lead. Lead-induced immunosuppression occurs at low dosages that induce no evident toxicity and, therefore, may be detrimental to the health of animals and perhaps of humans. The data accumulated to date provide good evidence that lead affects immunity, but additional studies are necessary to elucidate the actual mechanism by which lead exerts its immunosuppressive action. Knowledge of the effects of lead on the immune system of man is lacking and must be properly ascertained in order to determine permissible levels for human exposure. However, since this chemical affects immunity in laboratory animals and is immunosuppressive at very low dosages, its potential serious effects in man should be carefully considered.

12.9 EFFECTS OF LEAD ON OTHER ORGAN SYSTEMS

12.9.1 The Cardiovascular System

Lead has long been reported to be associated with cardiovascular effects, at least at high exposure levels. Some of the older literature bearing on this subject is reviewed here. In addition, because one of the the best understood pathophysiologic mechanisms of hypertension in humans is that resulting from renal disease, some clinical evidence linking lead-induced renal effects to hypertension is discussed in Section 12.5.3.5. (A more detailed discussion of lead-hypertension relationships, focusing on recently completed studies, can be found in an Addendum to this document.)

In regard to some of the older literature on lead's cardiovascular effects, Dingwall-Fordyce and Lane (1963) reported a marked increase in the cerebrovascular mortality rate among heavily exposed lead workers as compared with the normal expected rate. These workers were exposed to lead during the first quarter of this century when working conditions were quite bad. There has been no similar increase in the mortality rate reported for men employed in recent times.

Cardiovascular structural and functional changes have been noted for both adults and children with acute lead poisoning, but to date the extent of such studies has been limited. For example, cases have been described with regard to effects on the myocardium of children, always with clinical signs of poisoning. There is, of course, the possibility that the coexistence of lead poisoning and myocarditis is coincidental; but in many cases in which encephalopathy was present, the electrocardiographic abnormalities disappeared with chelation therapy, suggesting that lead may have indeed been the original etiological factor (Freeman, 1965; Myerson and Eisenhauer, 1963; Silver and Rodriguez-Torres, 1968). Silver and Rodriguez-Torres (1968) noted abnormal electrocardiograms in 21 of 30 children (70 percent) having symptoms of lead toxicity. After chelation therapy, the electrocardiograms remained abnormal in only four (13 percent) of the patients. In a review of five fatal cases of lead poisoning in young children, degenerative changes in heart muscle were reported to be the proximate cause of death (Kline, 1960). It is not clear that such morphological changes are a specific response to lead intoxication. Kósmider and Petelenz (1962) examined 38 adults over 46 years of age with chronic lead poisoning. They found that 66 percent had electrocardiographic changes, a rate that was four times the expected rate for that age group.

Electron microscopy of the myocardium of lead-intoxicated rats (Asokan, 1974) and mice (Khan et al., 1977) has shown diffuse degenerative changes. The susceptibility of the myocardium to toxic effects of lead was supported by in vitro studies in rat mitochondria by Parr and Harris (1976). These investigators found that the rate of Ca^{2+} removal by rat heart mitochondria is decreased by 1 nmol Pb/mg protein. Kopp and coworkers have demonstrated depression of contractility, isoproterenol responsiveness, and cardiac protein phosphorylation

(Kopp et al., 1980a), as well as high-energy phosphate levels (Kopp et al., 1980b) in hearts of lead-fed rats. Similarly, persistent increased susceptibility to norepinephrine-induced arrhythmias has been observed in rats fed lead during the first three weeks of life (Hejtmancik and Williams, 1977, 1978, 1979a,b; Williams et al., 1977a,b).

The cardiovascular effects of lead in conjunction with cadmium have been studied in rats following chronic low-level exposure by Perry and coworkers (Perry and Erlanger, 1978; Perry et al., 1979; Kopp et al., 1980a,b). Perry and Erlanger (1978) exposed female weanling Long-Evans rats to cadmium, lead, or cadmium plus lead (as acetate salts) at concentrations of 0.1, 1.0, or 5.0 ppm in deionized drinking water for up to 18 months. These authors reported statistically significant increases in systolic blood pressure for both cadmium and lead in the range of 15-20 mm Hg. Concomitant exposure to both cadmium and lead usually doubled the pressor effects of either metal alone. A subsequent study (Kopp et al., 1980a) using weanling female Long-Evans rats exposed to 5.0 ppm cadmium, lead, or lead plus cadmium in deionized drinking water for 15 or 20 months showed similar pressor effects of these two metals, alone or in combination, on systolic blood pressure. Electrocardiograms performed on these rats demonstrated statistically significant prolongation of the mean PR interval. Bundle electrograms also showed statistically significant prolongations. Other parameters of cardiac function were not markedly affected. Phosphorus-31 nuclear magnetic resonance (NMR) studies conducted on perchloric acid extracts of liquid nitrogen-frozen cardiac tissue from these animals disclosed statistically significant reductions in adenosine triphosphate (ATP) levels and concomitant increases in adenosine diphosphate (ADP) levels. Cardiac glycerol 3-phosphorylcholine (GPC) was also found to be significantly reduced using this technique, indicating a general reduction of tissue high-energy phosphates by lead or cadmium. Pulse-labeling studies using ^{32}P demonstrated decreased incorporation of this isotope into myosin light-chain (LC-2) in all lead or cadmium treatment groups relative to controls.

The results of this group of studies indicate that prolonged low-dose exposure to lead (and/or cadmium) reduces tissue concentrations of high-energy phosphates in rat hearts and suggest that this effect may be responsible for decreased myosin LC-2 phosphorylation and subsequent reduced cardiac contractility. Other experiments by these authors (Kopp et al., 1980b) were also conducted on isolated perfused hearts of weanling female Long-Evans rats exposed to cadmium, lead, or lead plus cadmium in deionized drinking water at concentrations of 50 ppm for 3-15 months. Incorporation of ^{32}P into cardiac proteins was studied following perfusion on inotropic perfusate containing isoproterenol at a concentration of 7×10^{-7} M. Data from these studies showed a statistically significant reduction in cardiac active tension in hearts from cadmium- or lead-treated rats. Incorporation of ^{32}P was also found to be signficantly reduced in myosin LC-2 proteins. The authors suggested that the observed decrease in LC-2 phosphorylation could be involved in the observed decrease in cardiac active tension in lead- or cadmium-treated rats.

There are conflicting reports regarding whether lead can cause atherosclerosis in experimental animals. Scroczynski et al. (1967) observed increased serum lipoprotein and cholesterol levels and cholesterol deposits in the aortas of rats and rabbits receiving large doses of lead. On the other hand, Prerovská (1973), using similar doses of lead given over an even longer period of time, did not produce atherosclerotic lesions in rabbits.

Makasev and Krivdina (1972) observed a two-phase change in the permeability of blood vessels (first increased, then decreased permeability) in rats, rabbits, and dogs that received a solution of lead acetate. A phase change in the content of catecholamines in the myocardium and in the blood vessels was observed in subacute lead poisoning in dogs (Mambeeva and Kobkova, 1969). This effect appears to be a link in the complex mechanism of the cardiovascular pathology of lead poisoning.

12.9.2 The Hepatic System

The effect of lead poisoning on liver function has not been extensively studied. In a study of 301 workers in a lead-smelting and refining facility, Cooper et al. (1973) found an increase in serum glutamic oxaloacetic transaminase (SGOT) activity in 11.5 percent of subjects with blood lead levels below 70 μg/dl, in 20 percent of those with blood lead levels of about 70 μg/dl, and in 50 percent of the workers with blood lead levels of about 100 μg/dl. The correlation ($r = 0.18$) between blood lead levels and SGOT was statistically significant. However, there must also have been exposure to other metals, e.g., cadmium, since there was a zinc plant in the smelter. In lead workers with moderate effects on the hematopoietic system and no obvious renal signs, SGOT was not increased compared with controls on repeated examinations (Hammond et al., 1980). In most studies on lead workers, tests for liver function are not included.

The liver is the major organ for the detoxification of drugs. In Section 12.3.1.3 it is mentioned that exposure to lead may cause altered drug detoxification rates as a result of interference with the formation of heme-containing cytochrome P-450, which is part of the hepatic mixed function oxidase system. This enzyme system is involved in the hepatic biotransformation of medicaments, hormones, and many environmental chemicals (Remmer et al., 1966). Whereas a decrease in drug-metabolizing activity clearly has been demonstrated in experimental animals given large doses of lead resulting in acute toxicity, the evidence for effects of that type in humans is less consistent. Alvares et al. (1975) studied the effect of lead exposure on drug metabolism in children. There was no difference between two normal children and eight children with biochemical signs of lead toxicity as far as their capacity to metabolize two test drugs, antipyrine and phenylbutazone. In two acutely poisoned children in whom blood levels of lead exceeded 60 µg/dl, antipyrine half-lives were significantly longer than normal, and therapy with EDTA led to biochemical remission of the disease and restoration of deranged drug metabolism toward normal. One of the "normal" children in this study had a blood lead level of 40 µg/dl, but normal ALA-D and EP values. No data were given on the analytical methods used for indices of lead exposure. Furthermore, the age of the children varied from 1 to 7.5 years, which is significant because, as pointed out by the authors, drug detoxification is age-dependent.

Meredith et al. (1977) demonstrated enhanced hepatic metabolism of antipyrine in lead-exposed workers (PbB: 77-195 µg/dl) following chelation therapy. The significance of this evidence of restored hepatic mixed oxidase function is, however, unclear because the pretreatment antipyrine biologic half-life and clearance were not significantly different in lead-exposed and control subjects. Moreover, there were more heavy smokers among the lead-exposed workers than controls. Smoking increases the drug-metabolizing capacity and may thus counteract the effects of lead. Also, the effect of chelation on antipyrine metabolism in non-exposed control subjects was not determined.

Hepatic drug metabolism was studied by Alvares et al. (1976) in eight adult patients showing marked effects of chronic lead intoxication on the erythropoietic system. The plasma elimination rate of antipyrine, which, as noted above, is a drug primarily metabolized by hepatic microsomal enzymes, was determined in eight subjects prior to and following chelation therapy. In seven of eight subjects, chelation therapy shortened the antipyrine half-lives, but the effect was minimal. The authors concluded that chronic lead exposure results in significant inhibition of the heme biosynthetic pathway without causing significant changes in enzymatic activities associated with hepatic cytochrome P-450.

A confounding factor in the above three studies may be that treatment with EDTA causes an increase in the glomerular filtration rate (GFR) if it has been reduced by lead (Section 12.5.3.3). This may cause a decrease in the half-lives of drugs. There are, however, no data on the effect of chelating agents on GFR in children or adults with moderate signs of lead toxicity.

In 11 children with blood lead levels between 43 and 52 µg/dl, Saenger et al. (1981) found a decrease in 24-hour urinary 6-beta-hydroxycortisol excretion that correlated closely ($r = 0.85$, $p < 0.001$) with a standardized EDTA lead-mobilization test (1000 mg EDTA/m^2 body surface area). This glucocorticoid metabolite is produced by the same hepatic microsomal mixed-function oxidase system that hydroxylates antipyrine. The authors suggest that the depression of 6-beta-hydroxylation of cortisol in the liver may provide a non-invasive method for assessing body lead stores in children (Saenger et al., 1981).

In a few animal studies, special attention has been paid to morphological effects of lead on the liver. Ledda-Columbano et al. (1983) investigated hepatic cell proliferation in male Wistar rats given 1,4, or 9, i.v. injections of lead nitrate (5 μmol/100 g body weight) at 10-day intervals. Although body weight was not significantly reduced and liver cell deaths did not increase, liver weight and DNA activity were both significantly increased. This work confirmed earlier findings by Columbano et al. (1983) that a single dose of lead nitrate (10 μmol/100 g) stimulated hepatic DNA synthesis in rats. Although the authors noted that cell proliferation could have been due to an adaptive mechanism, such proliferation could also have significant implications for liver carcinogenesis. More recently these findings were replicated and extended by Dessi et al. (1984), who observed a twofold increase in relative liver weight in rats 48 hours after an i.v. injection of lead nitrate (10 μmol/100 g). The investigators also found various indications of significantly increased cholesterol synthesis and glucose-6-phosphate dehydragenase, both of which were seen as consistent with the hyperplasia induced by lead.

White (1977) gave eight beagle dogs oral doses of lead carbonate, 50-100 mg Pb/kg b.w., for 3-7 weeks. Lead concentrations were not measured in blood or tissues. Morphological changes were noted in two dogs exposed from 5 weeks of age to 50 mg/kg. Changes in enzyme activities were found in most exposed animals; for example, some dehydrogenases showed increased activity after short exposure and decreased activity after longer exposures, mainly in animals with weight losses. The small number of animals and the absence of data on lead concentrations makes it impossible to use these results for risk evaluations.

Hoffmann et al. (1974) noted moderate to marked morphological changes in baboon livers after a single intravenous injection of large doses of lead acetate (25 mg/kg b.w.). It can be concluded that effects on the liver may be expected to occur only at high exposure levels. If effects on more sensitive systems, viz., the nervous and hematopoietic systems, are prevented, no adverse effects should be noted in the liver.

12.9.3 The Gastrointestinal System

Colic is usually a consistent early symptom of lead poisoning, warning of much more serious effects that are likely to occur with continued or more intense lead exposure. Although most commonly seen in industrial exposure cases, colic is also a lead-poisoning symptom present in infants and young children.

Beritic (1971) examined 64 men suffering from abdominal colic due to lead intoxication through occupational exposure. The diagnosis of lead colic was based on the occurrence of severe attacks of spasmodic abdominal pain accompanied by constipation, abnormally high coproporphyrinuria, excessive basophilic stippling, reticulocytosis, and some degree of anemia (all clinical signs of lead poisoning). Thirteen of the 64 patients had blood lead levels of 40-80 μg/dl upon admission. However, the report did not indicate how recently the patients' exposures had been terminated or provide other details of their exposure histories.

A more recent report by Dahlgren (1978) focused on the gastrointestinal symptoms of lead smelter workers whose blood lead levels were determined within two weeks of the termination of their work exposure. Of 34 workers with known lead exposure, 27 (79 percent) complained of abdominal pain, abnormal bowel movements, and nausea. Fifteen of the 27 had abdominal pain for more than 3 months after removal from the exposure to lead. The mean (± SD) blood lead concentration for this group of 15 was 70 (± 4) μg/dl. There was, however, no correlation between severity of symptoms and blood lead levels, as those experiencing stomach pain for less than 3 months averaged 68 (± 9) μg/dl and the remaining 7 workers, reporting no pain at all, averaged 76 (± 9) μg/dl.

Haenninen et al. (1979) assessed the incidence of gastrointestinal symptoms in 45 workers whose blood lead levels had been regularly monitored throughout their exposure and had never exceded 69 μg/dl. A significant association between gastrointestinal symptoms (particularly epigastric pain) and blood lead level was reported. This association was more pronounced in subjects whose maximal blood lead levels had reached 50-69 μg/dl, but was also noted in those whose blood lead levels were below 50 μg/dl.

Other occupational studies have also suggested a relationship between lead exposure and gastrointestinal symptoms (Lilis et al., 1977; Irwig et al., 1978a,b; Fischbein et al., 1979, 1980). For demonstrating such a relationship, however, the most useful measure of internal exposure has not necessarily been blood lead concentrations. Fischbein et al. (1980) surveyed a cross-section of New York City telephone cable splicers exposed to lead in the process of soldering cables. Of the 90 workers evaluated, 19 (21 percent) reported gastrointestinal symptoms related to lead colic. The difference between mean blood lead levels in those reporting GI symptoms and those not reporting such symptoms (30 versus 27 μg/dl) was not statistically significant. However, mean zinc protoporphyrin concentrations (67 versus 52 μg/dl) were significantly different ($p < 0.02$)

Limited experimental work has been devoted to gastrointestinal function either in humans (Lerza and Fierro, 1958; Mungo and Sessa, 1960) or animals (Mambeeva, 1963; Cory-Slechta et al., 1980). A recent study of chronically lead-exposed rats by Walsh and Ryden (1984) indicated that concentrations of lead sufficient to cause renal and hematological toxicity did not appreciably affect gastrointestinal transit.

Although gastrointestinal symptoms of lead exposure are clinically evident in frank lead intoxication and may even be present when blood lead levels approach the 30-80 μg/dl range, there is currently insufficient information to establish a clear dose-effect relationship for the general population at ambient exposure levels.

12.9.4 The Endocrine System

Some evidence exists for other effects of lead on the endocrine system, at least at high levels of lead exposure. Lead is thought, for example, to decrease thyroid function in man and experimental animals. Porritt (1931) suggested that lead dissolved from lead pipes by soft water was the cause of hypothyroidism in individuals living in southwest England. Later, Kremer and Frank (1955) reported the simultaneous occurrence of myxedema and plumbism in a house painter. Monaenkova (1957) observed impaired concentration of ^{131}I by thyroid glands in 10 of 41 patients with industrial plumbism. Subsequently, Zel'tser (1962) showed that *in vivo* ^{131}I uptake and thyroxine synthesis by rat thyroid were decreased by lead when doses of 2 and 5 percent lead acetate solution were administered. Robins et al. (1983) reported a clinical study of 12 workers with blood lead levels of 44 µg/dl or above. Seven of the workers showed low serum thyroxine and estimated free thyroxine levels. In the same report, the authors also presented results of a cross-sectional study of 47 workers in which both of these indices of thyroid function were negatively related to blood lead levels. The effects were more pronounced in black men than in white men. Refowitz (1984) reported that he was unable to corroborate the findings of Robins et al. (1983). However, his regression plots of similar thyroid function indices consistently showed negative relationships with blood lead levels in 58 workers. Although these results did not achieve statistical significance in Refowitz's (1984) analysis, they suggested a stronger negative relationship between thyroxine and blood lead levels in white workers than did the data of Robins et al. (1983).

Uptake of ^{131}I, sometimes decreased in men with lead poisoning, can be offset by treatment with thyroid-stimulating hormone (TSH) (Sandstead et al., 1969; Sandstead, 1967). Lead may act to depress thyroid function by inhibiting thiol groups or by displacing iodine in a protein sulfonyl iodine carrier (Sandstead, 1967), and the results suggest that excessive lead may act at both the pituitary and the thyroid gland itself to impair thyroid function. None of these effects on the thyroid system, however, have been demonstrated to occur in humans at blood lead levels below 30-40 µg/dl.

Sandstead et al. (1970a) studied the effects of lead intoxication on pituitary and adrenal function in man and found that it may produce clinically significant hypopituitarism in some. The effects of lead on adrenal function were less consistent, but some of the patients showed a decreased responsiveness to an inhibitor (metapyrone) of 11-beta-hydroxylation in the synthesis of cortisol. This suggests a possible impact of lead on pituitary-adrenal hormone functions. That excessive oral ingestion of lead may in fact result in pathological changes in the pituitary-adrenal axis is also supported by other reports (Murashov, 1966; Pines, 1965) of lead-induced decreased metapyrone responsiveness, a depressed pituitary reserve, and decreased immunoreactive adrenocorticotrophic hormone (ACTH). These same events may also affect adrenal gland function inasmuch as decreased urinary excretion of 17-hydroxycorticosteroids was observed in these patients. Furthermore, suppression of responsiveness to exogenous ACTH in the zona fasciculata of the adrenal cortex has been reported in lead-poisoned subjects (Makotchenko, 1965), and impairment of the zona glomerulosa of the adrenal cortex has also been suggested (Sandstead et al., 1970b). Once again, however, none of these effects on adrenal hormone function have been shown to occur at blood lead levels as low as 30-40 µg/dl.

Other studies provide evidence suggestive of lead exposure effects on endocrine systems controlling reproductive functions (see also Section 12.6). For example, evidence of abnormal luteinizing hormone (LH) secretory dynamics was found in secondary lead smelter workers (Braunstein et al., 1978). Reduced basal serum testosterone levels with normal basal LH levels, but a diminished rise in LH following stimulation, indicated suppression of hypothalamic-pituitary function. Testicular biopsies in two lead-poisoned workmen showed peritubular fibrosis suggesting direct toxic effects of lead in the testes as well as effects at the hypothalamic-pituitary level. Lancranjan et al. (1975) also reported lead-related interference with male reproductive functions. Moderately increased lead absorption (blood lead mean = 52.8 µg/dl) among a group of 150 workmen who had long-term exposure to lead in varying degrees was said to result in gonadal impairment. The effects on the testes were believed to be direct, however, in that tests for hypothalamic-pituitary influence were negative.

In regard to effects of lead on ovarian function in human females, Panova (1972) reported a study of 140 women working in a printing plant for 1-2 months, where ambient air lead levels were <7 µg/m^3. Using a classification of various age groups (20-25, 26-35, and 36-40 yr) and type of ovarian cycle (normal, anovular, and disturbed lutein phase), Panova claimed that statistically significant differences existed between the lead-exposed and control groups in the age range 20-25 years. It should be noted that the report does not show the age distribution, the level of significance, or the data on specificity of the method used for classification. Also, Zielhuis and Wibowo (1976), in a critical review of the above study, concluded that the design of the study and presentation of data were such that it was difficult to evaluate the author's conclusion that chronic exposure to low air lead levels leads to disturbed ovarian function. Moreover, no consideration was given to the dust levels of lead, an important factor in print shops. Unfortunately, little else besides the above report exists in the literature in regard to assessing lead effects on human ovarian function or other factors affecting human female fertility. Studies offering firm data on maternal variables such as hormonal state, which is known to affect the ability of the pregnant woman to carry the fetus full-term, are also lacking, although certain studies do at least indicate that high-level lead exposure induces stillbirths and abortions (see Section 12.6).

An animal study by Petrusz et al. (1979) reports that orally administered lead can exert effects on pituitary and serum gonadotropins, which may represent one mechanism by which lead affects reproductive functions. The blood lead levels at which alterations in serum and pituitary follicle stimulating hormone were observed in neonatal rats, however, were well in excess of 100 μg/dl. (Evidence relating endocrine function to various recently reported lead-associated effects on human fetal and child development, including effects on growth and stature, is reviewed in an Addendum to this document.)

12.10 CHAPTER SUMMARY

12.10.1 Introduction

Lead has diverse biological effects in humans and animals. Its effects are seen at the subcellular level of organellar structures and processes as well as at the overall level of general functioning that encompasses all systems of the body operating in a coordinated, interdependent fashion. The present chapter not only categorizes and describes the various biological effects of lead but also attempts to identify the exposure levels at which such effects occur and the mechanisms underlying them. The dose-response curve for the entire range of biological effects exerted by lead is rather broad, with certain biochemical changes occurring at relatively low levels of exposure and perturbations in other systems, such as the liver, becoming detectable only at relatively high exposure levels. In terms of relative vulnerability to deleterious effects of lead, the developing organism generally appears to be more sensitive than the mature individual. Additional, quantitative examination of overall exposure-effect relationships for lead is presented in Chapter 13. It should be noted that lead has no known beneficial biological effects. Available evidence does not demonstrate that lead is an essential element.

12.10.2 Subcellular Effects of Lead

The biological basis of lead toxicity is its ability to bind to ligating groups in biomolecular substances crucial to various physiological functions, thereby interfering with these functions by, for example, competing with native essential metals for binding sites, inhibiting enzyme activity, and inhibiting or otherwise altering essential ion transport. These effects are modulated by the following: 1) the inherent stability of such binding sites for lead; 2) the compartmentalization kinetics governing lead distribution among body compartments, among tissues, and within cells; and 3) the differences in biochemical organization across cells and tissues due to their specific functions. Given the complexities introduced by items 2 and 3, it is not surprising that no single unifying mechanism of lead toxicity across all tissues in humans and experimental animals has yet been demonstrated.

Insofar as effects of lead on activity of various enzymes are concerned, many of the available studies concern *in vitro* behavior of relatively pure enzymes with marginal relevance to various effects *in vivo*. On the other hand, certain enzymes are basic to the effects of lead at the organ or organ system level, and discussion is best reserved for such effects in the summary sections below dealing with lead's effects on particular organ systems. This section is mainly concerned with organellar effects of lead, especially those which provide some rationale for lead toxicity at higher levels of biological organization. Particular emphasis is placed on the mitochondrion, because this organelle is not only affected by lead in numerous ways but has also provided the most data bearing on the subcellular effects of lead.

The critical target organelle for lead toxicity in a variety of cell and tissue types clearly is the mitochondrion, followed probably by cellular and intracellular membranes. The mitochondrial effects take the form of structural changes and marked disturbances in mitochondrial function within the cell, particularly in energy metabolism and ion transport. These effects in turn are associated with demonstrable accumulation of lead in mitochondria, both *in vivo* and *in vitro*. Structural changes include mitochondrial swelling in a variety of cell types as well as distortion and loss of cristae, which occur at relatively moderate lead levels. Similar changes have also been documented in lead workers across a range of exposures.

Uncoupled energy metabolism, inhibited cellular respiration using both succinate and nicotinamide adenine dinucleotide (NAD)-linked substrates, and altered kinetics of intracellular calcium have been demonstrated *in vivo* using mitochondria of brain and non-neural tissues. In some cases, the lead exposure level associated with such changes has been relatively low. Several studies document the relatively greater sensitivity of this organelle in young versus adult animals in terms of mitochondrial respiration. The cerebellum appears to be particularly sensitive, providing a connection between mitochondrial impairment and lead encephalopathy. Lead's impairment of mitochondrial function in the developing brain has also been consistently associated with delayed brain development, as indexed by content of various cytochromes. In the rat pup, ongoing lead exposure from birth is required for this effect to be expressed, indicating that such exposure must occur before, and is inhibitory to, the burst of oxidative metabolism activity that occurs in the young rat at 10-21 days postnatally.

In vivo lead exposure of adult rats also markedly inhibits calcium turnover in a cellular compartment of the cerebral cortex that appears to be the mitochondrion. This effect has been seen at a brain lead level of 0.4 μg/g. These results are consistent with a separate study showing increased retention of calcium in the brains of lead-dosed guinea pigs. Numerous reports have described the *in vivo* accumulation of lead in mitochondria of kidney, liver, spleen, and brain tissue, with one study showing that such uptake was slightly more than occurred in the cell nucleus. These data are not only consistent with deleterious effects of lead on mitochondria but are also supported by other investigations *in vitro*. Significant decreases in mitochondrial respiration *in vitro* using both NAD-linked and succinate substrates have been observed for brain and non-neural tissue mitochondria in the presence of lead at micromolar levels. There appears to be substrate specificity in the inhibition of respiration across different tissues, which may be a factor in differential organ toxicity. Also, a number of enzymes involved in intermediary metabolism in isolated mitochondria have been observed to undergo significant inhibition of activity with lead.

Of particular interest regarding lead's effects on isolated mitochondria are ion transport effects, especially in regard to calcium. Lead movement into brain and other tissue mitochondria involves active transport, as does calcium. Recent sophisticated kinetic analyses of desaturation curves for radiolabeled lead or calcium indicate that there is striking overlap in the cellular metabolism of calcium and lead. These studies not only establish the basis for the easy entry of lead into cells and cell compartments, but also provide a basis for lead's impairment of intracellular ion transport, particularly in neural cell mitochondria, where the capacity for calcium transport is 20-fold higher than even in heart mitochondria.

Lead is also selectively taken up in isolated mitochondria in vitro, including the mitochondria of synaptosomes and brain capillaries. Given the diverse and extensive evidence of lead's impairment of mitochondrial structure and function as viewed from a subcellular level, it is not surprising that these derangements are logically held to be the basis of dysfunction of heme biosynthesis, erythropoiesis, and the central nervous system. Several key enzymes in the heme biosynthetic pathway are intramitochondrial, particularly ferrochelatase. Hence, it is to be expected that entry of lead into mitochondria will impair overall heme biosynthesis, and in fact this appears to be the case in the developing cerebellum. Furthermore, relatively moderate levels of lead may be associated with its entry into mitochondria and consequent expressions of mitochondrial injury.

Lead exposure provokes a typical cellular reaction in humans and other species that has been morphologically characterized as a lead-containing nuclear inclusion body. While it has been postulated that such inclusions constitute a cellular protection mechanism, such a mechanism is an imperfect one. Other organelles, e.g., the mitochondrion, also take up lead and sustain injury in the presence of nuclear inclusion formations.

In theory, the cell membrane is the first organelle to encounter lead and it is not surprising that cellular effects of lead can be ascribed to interactions at cellular and intracellular membranes in the form of disturbed ion transport. The inhibition of membrane (Na^+,K^+)-ATPase of erythrocytes as a factor in lead-impaired erythropoiesis is noted elsewhere. Lead also appears to interfere with the normal processes of calcium transport across membranes of different tissues. In peripheral cholinergic synaptosomes, lead is associated with retarded release of acetylcholine owing to a blockade of calcium binding to the membrane, while calcium accumulation within nerve endings can be ascribed to inhibition of membrane (Na^+,K^+)-ATPase.

Lysosomes accumulate in renal proximal convoluted tubule cells of rats and rabbits given lead over a range of dosing. This also appears to occur in the kidneys of lead workers and seems to represent a disturbance in normal lysosomal function, with the accumulation of lysosomes being due to enhanced degradation of proteins because of the effects of lead elsewhere within the cell.

12.10.3. Effects of Lead on Heme Biosynthesis, Erythropoiesis, and Erythrocyte Physiology in Humans and Animals

The effects of lead on heme biosynthesis are well known because of their clinical prominence and the numerous studies of such effects in humans and experimental animals. The process of heme biosynthesis starts with glycine and succinyl-coenzyme A, proceeds through formation of protoporphyrin IX, and culminates with the insertion of divalent iron into the porphyrin ring to form heme. In addition to being a constituent of hemoglobin, heme is the prosthetic group of many tissue hemoproteins having variable functions, such as myoglobin, the P-450 component of the mixed-function oxygenase system, and the cytochromes of cellular energetics. Hence, disturbance of heme biosynthesis by lead poses the potential for multiple-organ toxicity.

In investigations of lead's effects on the heme synthesis pathway, most attention has been devoted to the following: (1) stimulation of mitochondrial delta-aminolevulinic acid synthetase (ALA-S), which mediates formation of delta-aminolevulinic acid (ALA); (2) direct inhibition of the cytosolic enzyme, delta-aminolevulinic acid dehydrase (ALA-D), which catalyzes formation of porphobilinogen from two units of ALA; and (3) inhibition of insertion of iron (II) into protoporphyrin IX to form heme, a process mediated by ferrochelatase.

Increased ALA-S activity has been found in lead workers as well as in lead-exposed animals, although an actual decrease in enzyme activity has also been observed in several experimental studies using different exposure methods. It appears, then, that the effect on ALA-S activity may depend on the nature of the exposure. Using rat liver cells in culture, ALA-S activity was stimulated *in vitro* at lead levels as low as 5.0 μM or 1.0 μg/g preparation. The increased activity was due to biosynthesis of more enzyme. The blood lead threshold for stimulation of ALA-S activity in humans, based on a study using leukocytes from lead workers, appears to be about 40 μg/dl. Whether this apparent threshold applies to other tissues depends on how well the sensitivity of leukocyte mitochondria mirrors that in other systems. The relative impact of ALA-S activity stimulation on ALA accumulation at lower lead exposure levels appears to be much less than the effect of ALA-D activity inhibition. ALA-D activity is significantly depressed at 40 μg/dl blood lead, the point at which ALA-S activity only begins to be affected.

Erythrocyte ALA-D activity is very sensitive to inhibition by lead. This inhibition is reversed by reactivation of the sulfhydryl group with agents such as dithiothreitol, zinc, or zinc and glutathione. Zinc levels that achieve reactivation, however, are well above physiological levels. Although zinc appears to offset the inhibitory effects of lead observed in animal studies and in human erythrocytes in vitro, lead workers exposed to both zinc and lead do not show significant changes in the relationship of ALA-D activity to blood lead when compared with workers exposed just to lead. Nor does the range of physiological zinc levels in nonexposed subjects affect ALA-D activity. In contrast, zinc deficiency in animals significantly inhibits ALA-D activity, with concomitant accumulation of ALA in urine. Because zinc deficiency has also been demonstrated to increase lead absorption, the possibility exists for the following dual effects of such deficiency on ALA-D activity: (1) a direct effect on activity due to reduced zinc availability; and (2) increased lead absorption leading to further inhibition of activity.

Erythrocyte ALA-D activity appears to be inhibited at virtually all blood lead levels measured so far, and any threshold for this effect in either adults or children remains to be determined. A further measure of this enzyme's sensitivity to lead is a report that rat bone marrow suspensions show inhibition of ALA-D activity by lead at a level of 0.1 μg/g suspension. Inhibition of ALA-D activity in erythrocytes apparently reflects a similar effect in other tissues. Hepatic ALA-D activity in lead workers was inversely correlated with erythrocyte activity as well as blood lead levels. Of significance are experimental animal data showing that (1) brain ALA-D activity is inhibited with lead exposure, and (2) this inhibition appears to occur to a greater extent in developing animals than in adults, presumably reflecting greater retention of lead in developing animals. In the avian brain, cerebellar ALA-D activity is affected to a greater extent than that of the cerebrum and, relative to lead concentration, shows inhibition approaching that occurring in erythrocytes.

Inhibition of ALA-D activity by lead is reflected by elevated levels of its substrate, ALA, in blood, urine, and soft tissues. Urinary ALA is employed extensively as an indicator of excessive lead exposure in lead workers. The diagnostic value of this measurement in pediatric screening, however, is limited when only spot urine collection is done; more satisfactory data are obtainable with 24-hr collections. Numerous independent studies document a direct correlation between blood lead and the logarithm of urinary ALA in human adults and children; the blood lead threshold for increases in urinary ALA is commonly accepted as 40 μg/dl. However, several studies of lead workers indicate that the correlation between urinary ALA and blood lead continues below this value; one study found that the slope of the dose-effect curve in lead workers depends on the level of exposure.

The health significance of lead-inhibited ALA-D activity and accumulation of ALA at lower lead exposure levels is controversial. The "reserve capacity" of ALA-D activity is such that only the level of inhibition associated with marked accumulation of the enzyme's substrate, ALA, in accessible indicator media may be significant. However, it is not possible to quantify at lower levels of lead exposure the relationship of urinary ALA to target tissue levels or to relate the potential neurotoxicity of ALA at any accumulation level to levels in indicator media. Thus, the blood lead threshold for neurotoxicity of ALA may be different from that associated with increased urinary excretion of ALA.

Accumulation of protoporphyrin in erythrocytes of lead-intoxicated individuals has been recognized since the 1930s, but it has only recently been possible to quantitatively assess the nature of this effect via development of sensitive, specific microanalysis methods. Accumulation of protoporphyrin IX in erythrocytes results from impaired placement of iron (II) in the porphyrin moiety in heme formation, an intramitochondrial process mediated by ferrochelatase. In lead exposure, the porphyrin acquires a zinc ion in lieu of native iron, thus forming zinc protoporphyrin (ZPP), which is tightly bound in available heme pockets for the life of the erythrocytes. This tight sequestration contrasts with the relatively mobile nonmetal, or free, erythrocyte protoporphyrin (FEP) accumulated in the congenital disorder erythropoietic protoporphyria.

Elevation of erythrocyte ZPP has been extensively documented as exponentially correlated with blood lead in children and adult lead workers and is currently considered one of the best indicators of undue lead exposure. Accumulation of ZPP only occurs in erythrocytes formed during lead's presence in erythroid tissue; this results in a lag of at least several weeks before its buildup can be measured. The level of ZPP accumulation in erythrocytes of newly employed lead workers continues to increase after blood lead has already reached a plateau. This influences the relative correlation of ZPP and blood lead in workers with short exposure histories. Also, the ZPP level in blood declines much more slowly than blood lead, even after removal from exposure or after a drop in blood lead. Hence, ZPP level appears to be a more reliable indicator of continuing intoxication from lead resorbed from bone.

The threshold for detection of lead-induced ZPP accumulation is affected by the relative spread of blood lead and corresponding ZPP values measured. In young children (<4 yr old), the ZPP elevation associated with iron-deficiency anemia must also be considered. In adults, numerous studies indicate that the blood lead threshold for ZPP elevation is about 25-30 μg/dl. In children 10-15 years old, the threshold is about 16 μg/dl; for this age group, iron deficiency is not a factor. In one study, children over 4 years old showed the same threshold, 15.5 μg/dl, as a second group under 4 years old, indicating that iron deficiency was not a factor in the study. At 25 μg/dl blood lead, 50 percent of the children had significantly elevated FEP levels (2 standard deviations above the reference mean FEP).

At blood lead levels below 30-40 μg/dl, any assessment of the EP-blood lead relationship is strongly influenced by the relative analytical proficiency of measurements of both blood lead and EP. The types of statistical analyses used are also important. In a recent detailed statistical study involving 2004 children, 1852 of whom had blood lead values below 30 μg/dl, segmental line and probit analysis techniques were employed to assess the dose-effect threshold and dose-response relationship. An average blood lead threshold for the effect using both statistical techniques was 16.5 μg/dl for the full group and for those subjects with blood lead below 30 μg/dl. The effect of iron deficiency was tested for and removed. Of particular interest was the finding that blood lead values of 28.6 and 35.7 μg/dl corresponded to EP elevations more than 1 or 2 standard deviations, respectively, above the reference mean in 50 percent of the children. Hence, fully half of the children had significant elevations of EP at blood lead levels around 30 μg/dl. From various reports, children and adult females appear to be more sensitive to lead's effects on EP accumulation at any given blood lead level; children are somewhat more sensitive than adult females.

Lead's effects on heme formation are not restricted to the erythropoietic system. Recent studies show that the reduction of serum 1,25-dihydroxyvitamin D seen with even low-level lead exposure is apparently the result of lead-induced inhibition of the activity of renal 1-hydroxylase, a cytochrome P-450 mediated enzyme. Reduction in activity of the hepatic enzyme tryptophan pyrrolase and concomitant increases in plasma tryptophan as well as brain tryptophan, serotonin, and hydroxyindoleacetic acid have been shown to be associated with lead-induced reduction of the hepatic heme pool. The heme-containing protein cytochrome P-450 (an integral part of the hepatic mixed-function oxygenase system) is affected in humans and animals by lead exposure, especially acute intoxication. Reduced P-450 content correlates with impaired activity of detoxifying enzyme systems such as aniline hydroxylase and aminopyrine demethylase. It is also responsible for reduced 6β-hydroxylation of cortisol in children having moderate lead exposure.

Studies of organotypic chick and mouse dorsal root ganglion in culture show that the nervous system has heme biosynthetic capability and that not only is such capability reduced in the presence of lead but production of porphyrinic material is increased. In the neonatal rat, chronic lead exposure resulting in moderately elevated blood lead is associated with retarded increases in the hemoprotein cytochrome C and with disturbed electron transport in the developing cerebral cortex. These data parallel effects of lead on ALA-D activity and ALA accumulation in neural tissue. When both of these effects are viewed in the toxicokinetic context of increased retention of lead in both developing animals and children, there is an obvious and serious potential for impaired heme-based metabolic function in the nervous system of lead-exposed children.

As can be concluded from the above discussion, the health significance of ZPP accumulation rests with the fact that it is evidence of impaired heme and hemoprotein formation in many tissues that arises from entry of lead into mitochondria. Such evidence for reduced heme synthesis is consistent with a great deal of data documenting lead-associated effects on mitochondria. The relative value of the lead-ZPP relationship in erythropoietic tissue as an index of this effect in other tissues hinges on the relative sensitivity of the erythropoietic system compared with other organ systems. One study of rats exposed over their lifetime to low levels of lead demonstrated that protoporphyrin accumulation in renal tissue was already significant at levels of lead exposure which produced little change in erythrocyte porphyrin levels.

Other steps in the heme biosynthesis pathway are also known to be affected by lead, although these have not been as well studied on a biochemical or molecular level. Coproporphyrin levels are increased in urine, reflecting active lead intoxication. Lead also affects the activity of the enzyme uroporphyrinogen-I-synthetase in experimental animal systems, resulting in an accumulation of its substrate, porphobilinogen. The erythrocyte enzyme has been reported to be much more sensitive to lead than the hepatic species, presumably accounting for much of the accumulated substrate. Unlike the case with experimental animals, lead-exposed humans show no rise in urinary porphobilinogen, which is a differentiating characteristic of lead intoxication versus the hepatic porphyrias. Ferrochelatase is an intramitochondrial enzyme, and impairment of its activity, either directly by lead or via impairment of iron transport to the enzyme, is evidence of the presence of lead in mitochondria.

Anemia is a manifestation of chronic lead intoxication and is characterized as mildly hypochromic and usually normocytic. It is associated with reticulocytosis, owing to shortened cell survival, and the variable presence of basophilic stippling. Its occurrence is due to both decreased production and increased rate of destruction of erythrocytes. In young children (<4 yr old), iron deficiency anemia is exacerbated by lead uptake, and vice versa. Hemoglobin production is negatively correlated with blood lead in young children, in whom iron deficiency may be a confounding factor, as well as in lead workers. In one study, blood lead values that were usually below 80 μg/dl were inversely correlated with hemoglobin content. In these subjects no iron deficiency was found. The blood lead threshold for reduced hemoglobin content is about 50 μg/dl in adults and somewhat lower (~40 μg/dl) in children.

The mechanism of lead-associated anemia appears to be a combination of reduced hemoglobin production and shortened erythrocyte survival due to direct cell injury. Lead's effects on hemoglobin production involve disturbances of both heme and globin biosynthesis. The hemolytic component to lead-induced anemia appears to be caused by increased cell fragility and increased osmotic resistance. In one study using rats, the hemolysis associated with vitamin E deficiency, via reduced cell deformability, was exacerbated by lead exposure. The molecular basis for increased cell destruction rests with inhibition of (Na^+, K^+)-ATPase and pyrimidine-5'-nucleotidase. Inhibition of the former enzyme leads to cell "shrinkage" and inhibition of the latter results in impaired pyrimidine nucleotide phosphorolysis and disturbance of the activity of the purine nucleotides necessary for cellular energetics.

In lead intoxication, the presence of both basophilic stippling and anemia with a hemolytic component is due to inhibition by lead of the activity of pyrimidine-5'-nucleotidase (Py-5-N), an enzyme that mediates the dephosphorylation of pyrimidine nucleotides in the maturing erythrocyte. Inhibition of this enzyme by lead has been documented in lead workers, lead-exposed children, and experimental animal models. In one study of lead-exposed children, there was a negative correlation between blood lead and enzyme activity, with no clear response threshold. A related report noted that, in addition, there was a positive correlation between cytidine phosphate and blood lead and an inverse correlation between pyrimidine nucleotide and enzyme activity.

The metabolic significance of Py-5-N inhibition and cell nucleotide accumulation is that they affect erythrocyte stability and survival as well as potentially affect mRNA and protein synthesis related to globin chain synthesis. Based on one study of children, the threshold for the inhibition of Py-5-N activity appears to be about 10 µg/dl blood lead. Lead's inhibition of Py-5-N activity and a threshold for such inhibition are not by themselves the issue. Rather, the issue is the relationship of such inhibition to a significant level of impaired pyrimidine nucleotide metabolism and the consequences for erythrocyte stability and function. The relationship of Py-5-N activity inhibition by lead to accumulation of its pyrimidine nucleotide substrate is analogous to lead's inhibition of ALA-D activity and accumulation of ALA.

Tetraethyl lead and tetramethyl lead, components of leaded gasoline, undergo transformation *in vivo* to neurotoxic trialkyl metabolites as well as further conversion to inorganic lead. Hence, one might anticipate that exposure to such agents may result in effects commonly associated with inorganic lead, particularly in terms of heme synthesis and erythropoiesis. Various surveys and case reports show that the habit of sniffing leaded gasoline is associated with chronic lead intoxication in children from socially deprived backgrounds in rural or remote areas. Notable in these subjects is evidence of impaired heme biosynthesis, as indexed by significantly reduced ALA-D activity. In several case reports of frank lead toxicity from habitual leaded gasoline sniffing, effects such as basophilic stippling in erythrocytes and significantly reduced hemoglobin have also been noted.

The role of lead-associated disturbances of heme biosynthesis as a possible factor in neurological effects of lead is of considerable interest due to the following: (1) similarities between classical signs of lead neurotoxicity and several neurological components of the congenital disorder acute intermittent porphyria; and (2) some of the unusual aspects of lead neurotoxicity. There are three possible points of connection between lead's effects on heme biosynthesis and the nervous system. Associated with both lead neurotoxicity and acute intermittent porphyria is the common feature of excessive systemic accumulation and excretion of ALA. In addition, lead neurotoxicity reflects, to some degree, impaired synthesis of heme and hemoproteins involved in crucial cellular functions; such an effect on heme is now known to be relevant within neural tissue as well as in non-neural tissue.

Available information indicates that ALA levels are elevated in the brains of lead-exposed animals and arise through in situ inhibition of brain ALA-D activity or through transport of ALA to the brain after formation in other tissues. ALA is known to traverse the blood-brain barrier. Hence, ALA is accessible to, or formed within, the brain during lead exposure and may express its neurotoxic potential.

Based on various in vitro and in vivo neurochemical studies of lead neurotoxicity, it appears that ALA can inhibit release of the neurotransmitter gamma-aminobutyric acid (GABA) from presynaptic receptors at which ALA appears to be very potent even at low levels. In an in vitro study, ALA acted as an agonist at levels as low as 1.0 μM ALA. This in vitro observation supports results of a study using lead-exposed rats in which there was inhibition of both resting and K^+-stimulated release of preloaded ^{3}H-GABA from nerve terminals. The observation that in vivo effects of lead on neurotransmitter function cannot be duplicated with in vitro preparations containing added lead is further evidence of an effect of some agent (other than lead) that acts directly on this function. Human data on lead-induced associations between disturbed heme synthesis and neurotoxicity, while limited, also suggest that ALA may function as a neurotoxicant.

A number of studies strongly suggest that lead-impaired heme production itself may be a factor in the toxicant's neurotoxicity. In porphyric rats, lead inhibits tryptophan pyrrolase activity owing to reductions in the hepatic heme pool, thereby leading to elevated levels of tryptophan and serotonin in the brain. Such elevations are known to induce many of the neurotoxic effects also seen with lead exposure. Of great interest is the fact that heme infusion in these animals reduces brain levels of these substances and also restores enzyme activity and the hepatic heme pool. Another line of evidence for the heme-basis of lead neurotoxicity is that mouse dorsal root ganglion in culture manifests morphological evidence of neural injury with rather low lead exposure, but such changes are largely prevented with co-administration of heme. Finally, studies also show that heme-requiring cytochrome C production is impaired along with operation of the cytochrome C respiratory chain in the brain when neonatal rats are exposed to lead.

Awareness of the interactions of lead and the vitamin D-endocrine system has been growing. A recent study has found that children with blood lead levels of 33-120 μg/dl showed significant reductions in serum levels of the hormonal metabolite 1,25-dihydroxyvitamin D ($1,25\text{-}(OH)_2D$). This inverse dose-response relationship was found throughout the range of measured blood lead values, 12-120 μg/dl, and appeared to be the result of lead's effect on the production of the vitamin D hormone. The 1,25-$(OH)_2D$ levels of children with blood lead levels of 33-55 μg/dl corresponded to the levels that have been observed in children with severe renal dysfunction. At higher blood lead levels (>62 μg/dl), the 1,25-$(OH)_2D$ values were similar to those that have been measured in children with various inborn metabolic disorders. Chelation therapy of the lead-poisoned children (blood lead levels >62 μg/dl) resulted in a return to normal 1,25-$(OH)_2D$ levels within a short period.

In addition to its well-known actions on bone remodeling and intestinal absorption of minerals, the vitamin D hormone has several other physiological actions at the cellular level. These include cellular calcium homeostasis in virtually all mammalian cells and associated calcium-mediated processes that are essential for cellular integrity and function. In addition, the vitamin D hormone has newly recognized functions that involve cell differentiation and essential immunoregulatory capacity. It is reasonable to conclude, therefore, that impaired production of 1,25-$(OH)_2D$ can have profound and pervasive effects on tissues and cells of diverse type and function throughout the body.

12.10.4 Neurotoxic Effects of Lead

An assessment of the impact of lead on human and animal neurobehavioral function raises a number of issues. Among the key points addressed here are the following: (1) the internal exposure levels, as indexed by blood lead levels, at which various neurotoxic effects occur; (2) the persistence or reversibility of such effects; and (3) populations that appear to be most susceptible to neural damage. In addition, the question arises as to the utility of using animal studies to draw parallels to the human condition.

12.10.4.1 Internal Lead Levels at which Neurotoxic Effects Occur. Markedly elevated blood lead levels are associated with the most serious neurotoxic effects of lead exposure (including severe, irreversible brain damage as indexed by the occurrence of acute or chronic encephalopathic symptoms, or both) in both humans and animals. For most adult humans, such damage typically does not occur until blood lead levels exceed 120 μg/dl. Evidence does exist, however, for acute encephalopathy and death occurring in some human adults at blood lead as low as 100 μg/dl. In children, the effective blood lead level for producing encephalopathy or death is lower, starting at approximately 80-100 μg/dl. It should be emphasized that, once encephalopathy occurs, death is not an improbable outcome, regardless of the quality of medical treatment available at the time of acute crisis. In fact, certain diagnostic or treatment procedures themselves may exacerbate matters and push the outcome toward fatality if the nature and severity of the problem are not diagnosed or fully recognized. It is also crucial to note the rapidity with which acute encephalopathic symptoms can develop or death can occur in apparently asymptomatic individuals or in those apparently only mildly affected by elevated lead body burdens. Rapid deterioration often occurs, with convulsions or coma suddenly appearing with progression to death within 48 hours. This strongly suggests that even in apparently asymptomatic individuals, rather severe neural damage probably exists at high blood lead levels even though it is not yet overtly manifested in obvious encephalopathic symptoms. This conclusion is further supported by numerous studies showing that overtly lead intoxicated children with high blood lead levels, but not observed to manifest acute encephalopathic symptoms, are permanently cognitively impaired, as are most children who survive acute episodes of frank lead encephalopathy.

Recent studies show that overt signs and symptoms of neurotoxicity (indicative of both CNS and peripheral nerve dysfunction) are detectable in some human adults at blood lead levels as low as 40-60 µg/dl, levels well below blood lead concentrations previously thought to be "safe" for adult lead exposures. In addition, certain electrophysiological studies of peripheral nerve function in lead workers indicate that slowing of nerve conduction velocities in some peripheral nerves is associated with blood lead levels as low as 30-50 µg/dl (with no clear threshold for the effect being evident). These results are indicative of neurological dysfunctions occurring at relatively low lead levels in non-overtly lead-intoxicated adults.

Other evidence confirms that neural dysfunctions exist in apparently asymptomatic children at similar or even lower levels of blood lead. The body of studies on low- or moderate-level lead effects on neurobehavioral functions in non-overtly lead-intoxicated children, as summarized in Table 12-2, presents an array of data pointing to that conclusion. At high exposure levels, several studies point toward average 5-point IQ decrements occurring in asymptomatic children at average blood levels of 50-70 µg/dl. Other evidence is indicative of average IQ decrements of about 4 points being associated with blood levels in a 30-50 µg/dl range. Below 30 µg/dl, the evidence for IQ decrements is quite mixed, with some studies showing no significant associations with lead once other confounding factors are controlled. Still, the 1-2 point differences in IQ generally seen with blood lead levels in the 15-30 µg/dl range are suggestive of lead effects that are often dwarfed by other socio-hereditary factors. Moreover, a highly significant linear relationship between IQ and blood lead over the range of 6 to 47 µg/dl found in low-SES Black children indicates that IQ effects may be detected without evident threshold even at these low levels, at least in this population of children. In addition, other behavioral (e.g., reaction time, psychomotor performance) and electrophysiological (altered EEG patterns, evoked potential measures, and peripheral nerve conduction velocities) are consistent with a dose-response function relating neurotoxic effects to lead exposure levels as low as 15-30 µg/dl and possibly lower. Although the comparability of blood lead concentrations across species is uncertain (see discussion below), studies show neurobehavioral effects in rats and monkeys at maximal blood lead levels below

20 µg/dl; some studies demonstrate residual effects long after lead exposure has terminated and blood lead levels have returned to approximately normal levels.

Timing, type, and duration of exposure are important factors in both animal and human studies. It is often uncertain whether observed blood lead levels represent the levels that were responsible for observed behavioral deficits or electrophysiological changes. Monitoring of lead exposures in pediatric subjects in all cases has been highly intermittent or nonexistent during the period of life preceding neurobehavioral assessment. In most studies of children, only one or two blood lead values are provided per subject. Tooth lead may be an important cumulative exposure index, but its modest, highly variable correlation to blood lead, FEP, or external exposure levels makes findings from various studies difficult to compare quantitatively. The complexity of the many important covariates and their interaction with dependent variable measures of modest validity, e.g., IQ tests, may also account for many of the discrepancies among the different studies.

12.10.4.2 The Question of Irreversibility. Little research on humans is available on persistence of effects. Some work suggests that mild forms of peripheral neuropathy in lead workers may be reversible after termination of lead exposure, but little is known regarding the reversibility of lead effects on central nervous system function in humans. A two-year follow-up study of 28 children of battery factory workers found a continuing relationship between blood lead levels and altered slow wave voltage of cortical slow wave potentials indicative of persisting CNS effects of lead, and a five-year follow-up of some of the same children revealed the presence of altered brain stem auditory evoked potentials. Current population studies, however, will have to be supplemented by longitudinal studies of the effects of lead on development in order to address the issue of the reversibility or persistence of the neurotoxic effects of lead in humans more satisfactorily. (See the Addendum to this document for a discussion of recent results from prospective studies linking perinatal lead exposure to postnatal mental development.)

Various animal studies provide evidence that alterations in neurobehavioral function may be long-lived, with such alterations being evident long after blood lead levels have returned to control levels. These persistent effects have been demonstrated in monkeys as well as rats under a variety of learning performance test paradigms. Such results are also consistent with morphological, electrophysiological, and biochemical studies on animals that suggest lasting changes in synaptogenesis, dendritic development, myelin and fiber tract formation, ionic mechanisms of neurotransmission, and energy metabolism.

12.10.4.3 Early Development and the Susceptibility to Neural Damage. On the question of early childhood vulnerability, the neurobehavioral data are consistent with morphological and biochemical studies of the susceptibility of the heme biosynthetic pathway to perturbation by lead. Various lines of evidence suggest that the order of susceptibility to lead's effects is as follows: (1) young > adults and (2) female > male. Animal studies also have pointed to the perinatal period of ontogeny as a particularly critical time for a variety of reasons: (1) it is a period of rapid development of the nervous system; (2) it is a period when good nutrition is particularly critical; and (3) it is a period when the caregiver environment is vital to normal development. However, the precise boundaries of a critical period are not yet clear and may vary depending on the species and function or endpoint that is being assessed. One analysis of lead-exposed children suggests that differing effects on cognitive performance may be a function of the different ages at which children are subjected to neurotoxic exposures. Nevertheless, there is general agreement that human infants and toddlers below the age of three years are at special risk because of in utero exposure (see Addendum), increased opportunity for exposure because of normal mouthing behavior, and increased rates of lead absorption due to various factors, e.g., nutritional deficiences.

12.10.4.4 Utility of Animal Studies in Drawing Parallels to the Human Condition. Animal models are used to shed light on questions where it is impractical or ethically unacceptable to use human subjects. This is particularly true in the case of exposure to environmental toxins such as lead. In the case of lead, it has been effective and convenient to expose developing animals via their mothers' milk or by gastric gavage, at least until weaning. In many studies, exposure was continued in the water or food for some time beyond weaning. This approach simulates at least two features commonly found in human exposure: oral intake and exposure during early development. The preweaning period in rats and mice is of particular relevance in terms of parallels with the first two years or so of human brain development.

Studies using rodents and monkeys have provided a variety of evidence of neurobehavioral alteration induced by lead exposure. In most cases these effects suggest impairment in "learning," i.e., the process of appropriately modifying one's behavior in response to information from the environment. Such behavior involves the ability to receive, process, and remember information in various forms. Some studies indicate behavioral alterations of a more basic type, such as delayed development of certain reflexes. Other evidence suggests changes affecting rather complex behavior in the form of social interactions.

Most of the above effects are evident in rodents and monkeys with blood lead levels exceeding 30 μg/dl, but some effects on learning ability are apparent even at maximum blood lead exposure levels below 20 μg/dl. Can these results with animals be generalized to humans? Given differences between humans, rats, and monkeys in heme chemistry, metabolism, and other aspects of physiology and anatomy, it is difficult to state what constitutes an equivalent internal exposure level (much less an equivalent external exposure level). For example, is a blood lead level of 30 μg/dl in a suckling rat equivalent to 30 μg/dl in a three-year-old child? Until an answer is available for this question, i.e., until the function describing the relationship of exposure indices in different species is available, the utility of animal models for deriving dose-response functions relevant to humans will be limited.

Questions also exist regarding the comparability of neurobehavioral effects in animals with human behavior and cognitive function. One difficulty in comparing behavioral endpoints such as locomotor activity is the lack of a consistent operational definition. In addition to the lack of standardized methodologies, behavior is notoriously difficult to "equate" or compare meaningfully across species because behavioral analogies do not demonstrate behavioral homologies. Thus, it is improper to assume, without knowing more about the responsible underlying neurological structures and processes, that a rat's performance on an operant conditioning schedule or a monkey's performance on a stimulus discrimination task corresponds to a child's performance on a cognitive function test. Nevertheless, interesting parallels in hyper-reactivity and increased response variability do exist between different species, and deficits in performance on various tasks are indicative of altered CNS functions, which are likely to parallel some type of altered CNS function in humans as well.

In terms of morphological findings, there are reports of hippocampal lesions in both lead-exposed rats and humans that are consistent with a number of behavioral findings suggesting an impaired ability to respond appropriately to altered contingencies for rewards. That is, subjects tend to persist in certain patterns of behavior even when changed conditions make the behavior inappropriate. Other morphological findings in animals, such as demyelination and glial cell decline, are comparable to human neuropathologic observations mainly at relatively high exposure levels.

Another neurobehavioral endpoint of interest in comparing human and animal neurotoxicity of lead is electrophysiological function. Alterations of electroencephalographic patterns and cortical slow wave voltage have been reported for lead-exposed children, and various electrophysiological alterations both *in vivo* (e.g., in rat visual evoked response) and *in vitro* (e.g., in frog miniature endplate potentials) have also been noted in laboratory animals. At this time, however, these lines of work have not converged sufficiently to allow for strong conclusions regarding the electrophysiological aspects of lead neurotoxicity.

Biochemical approaches to the experimental study of lead's effects on the nervous system have generally been limited to laboratory animal subjects. Although their linkage to human neurobehavioral function is at this point somewhat speculative, such studies do provide insight to possible neurochemical intermediaries of lead neurotoxicity. No single neurotransmitter system has been shown to be particularly sensitive to the effects of lead exposure; lead-induced alterations have been demonstrated in various neurotransmitters, including dopamine, norepinephrine, serotonin, and γ-aminobutyric acid. In addition, lead has been shown to have subcellular effects in the central nervous system at the level of mitochondrial function and protein synthesis.

Given the above-noted difficulties in formulating a comparative basis for internal exposure levels among different species, the primary value of many animal studies, particularly *in vitro* studies, may be in the information they can provide on basic mechanisms involved in lead neurotoxicity. A number of *in vitro* studies show that significant, potentially deleterious effects on nervous system function occur at *in situ* lead concentrations of 5 μM and possibly lower, suggesting that no threshold may exist for certain neurochemical effects of lead on a subcellular or molecular level. The relationship between blood lead levels and lead concentrations at such extra- or intracellular sites of action, however, remains to be determined. Despite the problems in generalizing from animals to humans, both the animal and the human studies show great internal consistency in that they support a continuous dose-response functional relationship between lead and neurotoxic biochemical, morphological, electrophysiological, and behavioral effects.

12.10.5 Effects of Lead on the Kidney

It has been known for more than a century that kidney disease can result from lead poisoning. Identifying the contributing causes and mechanisms of lead-induced nephropathy has been difficult, however, in part because of the complexities of human exposure to lead and other nephrotoxic agents. Nevertheless, it is possible to estimate at least roughly the range of lead exposure associated with detectable renal dysfunction in both human adults and children. Numerous studies of occupationally exposed workers have provided evidence for lead-induced chronic nephropathy being associated with blood lead levels ranging from 40 to more than 100 μg/dl, and some are suggestive of renal effects possibly occurring even at levels as low as 30 μg/dl. In children, the relatively sparse evidence available points to the manifestation of nephropathy only at quite high blood lead levels (usually exceeding 100-120 μg/dl). The current lack of evidence for nephropathy at lower blood lead levels in children may simply reflect the greater clinical concern with neurotoxic effects of lead intoxication in children or, possibly, that much longer-term lead exposures are necessary to induce nephropathy. The persistence of lead-induced nephropathy in children also remains to be more fully investigated, although a few studies indicate that children diagnosed as being acutely lead poisoned experience lead nephropathy effects lasting throughout adulthood.

Parallel results from experimental animal studies reinforce the findings in humans and help illuminate the mechanisms underlying such effects. For example, a number of transient effects in human and animal renal function are consistent with experimental findings of reversible lesions such as nuclear inclusion bodies, cytomegaly, swollen mitochondria, and increased numbers of iron-containing lysosomes in proximal tubule cells. Irreversible lesions such as interstitial fibrosis are also well documented in both humans and animals following chronic exposure to high doses of lead. Functional renal changes observed in humans have also been confirmed in animal model systems with respect to increased excretion of amino acids and elevated serum urea nitrogen and uric acid concentrations. The inhibitory effects of lead exposure on renal blood flow and glomerular filtration rate are currently less clear in experimental model systems; further research is needed to clarify the effects of lead on these functional parameters in animals. Similarly, while lead-induced perturbation of the renin-angiotensin system has been demonstrated in experimental animal models, further research is needed to clarify the exact relationships among lead exposure (particularly chronic low-level exposure), alteration of the renin-angiotensin system, and hypertension in both humans and animals.

On the biochemical level, it appears that lead exposure produces changes at a number of sites. Inhibition of membrane marker enzymes, decreased mitochondrial respiratory function/cellular energy production, inhibition of renal heme biosynthesis, and altered nucleic acid synthesis are the most marked changes to have been reported. The extent to which these mitochondrial alterations occur is probably mediated in part by the intracellular bioavailability of lead, which is determined by its binding to high-affinity kidney cytosolic proteins and deposition within intranuclear inclusion bodies.

Among the questions remaining to be answered more definitively about the effects of lead on the kidneys is the lowest blood lead level at which renal effects occur. In this regard it should be noted that recent studies in humans have indicated that the EDTA lead-mobilization test is the most reliable technique for detecting persons at risk for chronic nephropathy; blood lead measurements are a less satisfactory indicator because they may not accurately reflect cumulative absorption some time after exposure to lead has terminated. Other questions include the following: Can a distinctive lead-induced renal lesion be identified either in functional or histologic terms? What biologic measurements are most reliable for the prediction of lead-induced nephropathy? What is the incidence of lead nephropathy in the general population as well as among specifically defined subgroups with varying exposure? What is the natural history of treated and untreated lead nephropathy? What is the mechanism of lead-induced hypertension and renal injury? What are the contributions of environmental and genetic factors to the appearance of renal injury due to lead? Conversely, the most difficult question of all may well be to determine the contribution of low levels of lead exposure to possible exacerbation of renal disease of non-lead etiologies.

12.10.6 Effects of Lead on Reproduction and Development

The most clear-cut data described in this section on reproduction and development are derived from studies employing high lead doses in laboratory animals. There is still a need for more critical research to evaluate the possible subtle toxic effects of lead on the fetus, using biochemical, ultrastructural, or behavioral endpoints. An exhaustive evaluation of lead-associated changes in offspring should include consideration of possible effects due to paternal lead burden as well. Neonatal lead intake via consumption of milk from lead-exposed mothers may also be a factor at times. Moreover, it must be recognized that lead's effects on reproduction may be exacerbated by other environmental factors (e.g., dietary influences, maternal hyperthermia, hypoxia, and co-exposure to other toxins).

There are currently no reliable data pointing to adverse effects in human offspring following lead exposure of fathers *per se*. Early studies of pregnant women exposed to high levels of lead indicated toxic, but not teratogenic, effects on the conceptus. Unfortunately, the collective human data regarding lead's effects on reproduction or *in utero* development currently do not lend themselves to accurate estimation of exposure-effect or no-effect levels. This is particularly true regarding effects on reproductive performance in women, which have not been well documented at low exposure levels. Still, prudence would argue for avoidance of lead exposures resulting in blood lead levels exceeding 25-30 µg/dl in pregnant women or women of child-bearing age in general, given the equilibration between maternal and fetal blood lead concentrations that occurs and the growing evidence for deleterious effects in young children as blood lead levels approach or exceed 25-30 µg/dl. Industrial exposure of men to lead at levels resulting in blood lead values of 40-50 µg/dl also appear to result in altered testicular function.

The paucity of human exposure data forces an examination of the animal studies for indications of threshold levels for effects of lead on the conceptus. It must be noted that the animal data are almost entirely derived from rodents. Based on these rodent data, it seems likely that fetotoxic effects have occurred in animals at chronic exposures to 600-800 ppm inorganic lead in the diet. Subtle effects appear to have been observed at 5-10 ppm in the drinking water, while effects of inhaled lead have been seen at levels of 10 mg/m^3. With multiple exposure by gavage, the lowest observed effect level is 64 mg/kg per day, and for exposure via injection, acute doses of 10-16 mg/kg appear effective. Since humans are most likely to be exposed to lead in their diet, air, or water, the data from other routes of exposure are of less value in estimating harmful exposures. Indeed, it appears that teratogenic effects occur in experimental animals only when the maternal dose is given by injection.

Although human and animal responses may be dissimilar, the animal evidence does document a variety of effects of lead exposure on reproduction and development. Measured or apparent changes in production of or response to reproductive hormones, toxic effects on the gonads, and toxic or teratogenic effects on the conceptus have all been reported. The animal data also suggest subtle effects on such parameters as metabolism and cell structure that should be monitored in human populations. Well-designed prospective human epidemiological studies involving large numbers of subjects are still needed (beyond the few currently available). Such data could clarify the relationship of exposure periods, exposure durations, and blood lead concentrations associated with significant effects and are needed for estimation of no-effect levels as well. (Recent studies, most of which are prospective epidemiological investigations, on the relationship between relatively low-level lead exposure and effects on fetal and child development, along with supporting experimental evidence on possible underlying mechanisms, are reviewed in an Addendum to this document.)

12.10.7 Genotoxic and Carcinogenic Effects of Lead

It is difficult to conclude what role lead may play in the induction of human neoplasia. Epidemiological studies of lead-exposed workers provide no definitive findings. However, statistically significant elevations in cancer of the respiratory tract and digestive system in workers exposed to lead and other agents warrant some concern. Since it is clear that lead acetate can produce renal tumors in some experimental animals, it seems reasonable to conclude that at least this particular lead compound should be regarded as a carcinogen and prudent to treat it as if it were also a human carcinogen (as concluded by the International Agency for Research on Cancer). However, this statement is qualified by noting that lead has been seen to increase tumorigenesis rates in animals only at relatively high concentrations, and therefore does not seem to be a potent carcinogen. *In vitro* studies further support the genotoxic and carcinogenic role of lead, but also indicate that lead is not potent in these systems.

12.10.8 Effects of Lead on the Immune System

Lead renders animals more susceptible to endotoxins and infectious agents. Host susceptibility and the humoral immune system appear to be particularly sensitive. As postulated in recent studies, the macrophage may be the primary immune target cell of lead. Lead-induced immunosuppression occurs in experimental animals at low lead exposures that, although not inducing overt toxicity, may nevertheless be detrimental to health. Available data provide good evidence that lead affects immunity, but additional studies are necessary to elucidate the actual mechanisms by which lead exerts its immunosuppressive action. Knowledge of the effects of lead on the human immune system is lacking and must be ascertained in order to determine permissible levels for human exposure. However, in view of the fact that lead affects immunity in laboratory animals and is immunosuppressive at very low dosages, its potential for serious effects in humans should be carefully considered.

12.10.9 Effects of Lead on Other Organ Systems

The cardiovascular, hepatic, gastrointestinal, and endocrine systems generally show signs of dysfunction mainly at relatively high lead exposure levels. Consequently, in most clinical and experimental studies, attention has been primarily focused on more sensitive and vulnerable target organs, such as the hematopoietic and nervous systems. However, some work does suggest that humans and animals show significant increases in blood pressure following chronic exposure to low levels of lead (see Addendum to this document for a detailed discussion of the relationship between blood lead and blood pressure and the possible biological mechanisms which may be responsible for this association). It should also be noted that overt gastrointestinal symptoms associated with lead intoxication have been observed to occur in lead workers at blood lead levels as low as 40-60 µg/dl. These findings suggest that effects on the gastrointestinal and cardiovascular systems may occur at relatively low exposure levels, but remain to be more conclusively demonstrated by further scientific investigations. Current evidence indicates that various endocrine processes may be affected by lead at relatively high exposure levels. Little information exists on endocrine effects at lower exposure levels, except for alterations in vitamin-D metabolism previously discussed as secondary to heme synthesis effects and occurring at blood lead levels ranging below 30 µg/dl to as low as 12 µg/dl. (Evidence relating endocrine function to various recently reported lead-associated effects on human fetal and child development, including effects on growth and stature, is reviewed in the Addendum to this document.)

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APPENDIX 12-A

SUMMARY OF PSYCHOMETRIC TESTS USED TO ASSESS COGNITIVE AND BEHAVIORAL DEVELOPMENT IN PEDIATRIC POPULATIONS

TABLE 12A. TESTS COMMONLY USED IN A PSYCHO-EDUCATIONAL BATTERY FOR CHILDREN

	Age range	Norms	Scores	Advantages	Disadvantages
General Intelligence Tests					
Stanford-Binet (Form L-M)	2 yrs - Adult	1972	1. Deviation IQ: Mean = 100 SD = 16 2. Mental Age Equivalent	1. Good reliability & validity 2. Predicts school performance 3. Covers a wide age range	1. Tests mostly verbal skills especially after 6 yrs 2. Does not give a profile of skills
Wechsler Preschool & Primary Scales of Intelligence (WPPSI)	4 - 6½ yrs Best for 5-yr-olds	1967	1. Deviation IQ: Mean = 100 SD = 15 2. Scaled Scores for 10 sub tests: Mean = 10 SD = 3	1. Good reliability & validity 2. Predicts school performance 3. Gives a profile of verbal & non-verbal skills. 4. Useful in early identification of learning disability	1. Narrow age range 2. Mentally retarded children find this a disproportionately difficult test
Wechsler Intelligence Scale for Children-Revised (WISC-R)	6 - 16 yrs	1974	1. Deviation IQ: Mean = 100 SD = 15 2. Scaled Scores for 10 subtests: Mean = 10 SD = 3	1. Good reliability & validity 2. Predicts school performance 3. Gives a profile of verbal and non-verbal skills 4. Useful in identification of learning disability	1. Gives a lower IQ than Stanford-Binet for normal and bright children
McCarthy Scales of Children's Abilities (MSCA)	2½ - 8½ yrs Best for ages 4 - 6	1972	1. General Cognitive Index: Mean = 100 SD = 16 2. Scaled scores for 5 subtests: mean = 50 SD = 10 Age equivalents can be derived.	1. Good reliability & validity 2. Good predictor of school performance 3. Useful in identification of learning disabilities when given with a WISC-R or Stanford-Binet 4. Gives good information for educational programming	1. Children score much lower than on WISC-R or Stanford-Binet 2. Narrow age range
Bayley Scales of Mental Development	2 - 30 mos.	1969	1. Standard scores (M = 100 SD = 16) 2. Mental Development Psychomotor Index	1. Norms are excellent 2. Satisfactory reliability and validity 3. Best measure of infant development	1. Not a good predictor of later functioning in average as in below average children

TABLE 12A. (continued)

	Age range	Norms	Scores	Advantages	Disadvantages
Slosson Intelligence Test	Infancy - 27 yrs	1963	1. Ratio IQ: is not related to general population	1. Good reliability & validity 2. Quick to administer. A good screening test	1. Many items taken from Stanford Binet 2. Responses require good language skills 3. Measures a narrow range of skills 4. A screening test: not to be used for classification or placement
Peabody Picture Vocabulary Test	2½ - 18 yrs	1959, rev. 1981 White, Middle class sample	1. Verbal IQ 2. Age equivalent	1. Easily administered 2. Does not require language or motor skills	1. Fair reliability and validity 2. Tests only receptive vocabulary 3. Lower class children score lower 4. Mentally Retarded children score higher than on other tests 5. Not to be used for classification or placement.
Visual-Motor Tests					
Frostig Developmental Test of Visual Perception	3 - 8 yrs & older learning disabled (L.D.) children	1963 White, middle class sample	1. Perceptual Quotient: Median = 100 Quartile Deviation = 10 2. Perceptual Age Equivalent 3. Scaled Scores for 5 subtests	1. Good reliability for L.D. children	1. Fair reliability for normal children 2. Poor Validity 3. No known relationship to reading or learning 4. Remedial program based on test of questionable value 5. Not useful in identifying children at risk for L.D.
Bender-Gestalt	4 yrs - Adult	1964 Normal and Brain-injured Children	1. Age equivalent	1. Easily administered 2. Long history of research makes it a good research tool	1. Fair reliability 2. Poor predictive and validity 3. Responses influenced by fatigue & variations in administration 4. No known relationship to reading or subtle neurological dysfunction
Beery-Buktenica Developmental Test of Visual Motor Integration (VMI)	2 - 15 yrs	1967	1. Age equivalent	1. Easily administered 2. Good normative sample	1. Moderate reliability and validity 2. Correlates better with mental age than chronological age

TABLE 12A. (continued)

	Age range	Norms	Scores	Advantages	Disadvantages
Educational Tests					
Wide Range Achievement Test (WRAT)	5 yrs - Adult	1976 Revised	1. Standard Score: mean = 100 SD = 15 2. Grade equivalent	1. Good reliability & validity Reading scores predict grade level 2. Tasks similar to actual work	1. Reading portion tests word recognition only 2. Responses require good organizational skills (could be an advantage)
Peabody Individual Achievement Test (PIAT)	5 - 18 yrs	1969	1. Standard Scores: Mean = 100 SD = 15 2. Grade equivalent 3. Age equivalent	1. Tests word recognition and 2. Breaks down skills into 5 areas	1. Moderate reliability. Low stability for Kindergarten 2. No data on predictive validity 3. A multiple choice test requiring child to recognize correct answer (could be an advantage). 4. Heavily loaded on verbal reasoning. 5. Factor structure changes with age.
Woodcock Reading Mastery Tests	Kgn - 12 grade	1971-72 adjusted for social class	1. Grade equivalent 2. Standard Score 3. Percentile Rank	1. Good reliability 2. Breakdown of reading skills useful diagnostically and in planning remediation 3. Easy to administer and score	1. No data on validity
Spache Diagnostic Reading Scales	1st - 8th grade	1972	1. Instructional level of reading (grade equivalent). 2. Independent level of reading. 3. Potential level of reading	1. Independent level score predicts gains following remediation 2. Good breakdown of reading skills	1. Fairly complex scoring 2. Moderate reliability 3. No good data on validity
Key Math Diagnostic Arithmetic Test	Pre-school - 6th grade	1971	1. Grade equivalent	1. Excellent breakdown of math skills 2. Easy to administer and score	1. Moderate reliability 2. No data on validity

TABLE 12A. (continued)

	Age range	Norms	Scores	Advantages	Disadvantages
Tests of Adaptive Functioning					
Vineland Social Maturity Scale	Birth - 25 yrs	1983 Revised	1. Social Quotient (Ratio) 2. Social Age Equivalent	1. Easily administered 2. Good reliability for normal and MR chidren	1. Poor norms 2. No data on validity 3. Items are limited past preschool years 4. Scores decrease with age for MR children
AAMD Adaptive Behavior Scale	3 yrs - Adult	1974 Institutionalized Retardates; Public School Children (1982)	1. Percentile Ranks 2. Scaled scores	1. Discriminates between EMR and regular classes 2. Useful for class placement and monitoring progress	1. Moderate reliability for independent living skills scale. Poor reliability for maladaptive behaviour scale. 2. Lengthy administration 3. Items & scoring are not behaviorally objective
Progress Assessment Chart of Social Development (PAC)	Birth - Adult	1976	No Scores	1. Useful for training and assessing progress 2. Gives profile of skills	1. No data on reliability or validity
Developmental Profile	Birth - 12 yrs	1972	1. Age equivalents in 5 5 areas 2. IQ equivalency (IQE)	1. Good reliability and validity. Excellent study of construct validity reported in manual. 2. Gives a profile of skills	1. IQE underestimates IQ of above average children, overestimates IQ of below average children.
Conners Rating Scale	3 yrs - 17 yrs	1978	1. Age equivalents	1. Most widely used measure of attention deficit disorder 2. Four factors: conduct problems; hyperactivity; inattentive-passive; hyperactivity index	1. Parents' ratings don't predict as well as teachers' ratings 2. Works best middle class children
Werry-Weiss-Peters Hyperactivity Scale	1 yr - 9 yrs	1974, 1977	1. Age equivalents	1. Good measure of hyperactivity 2. Seven Factors	1. Limited age range 2. Standardized on middle class children

13. EVALUATION OF HUMAN HEALTH RISKS ASSOCIATED WITH EXPOSURE TO LEAD AND ITS COMPOUNDS

13.1 INTRODUCTION

This chapter attempts to integrate, concisely, key information and conclusions discussed in preceding chapters into a coherent framework by which interpretation and judgments can be made concerning the risk to human health posed by present levels of lead contamination in the United States.

Towards this end, the chapter is organized into eight sections, each of which discusses one or more of the following major components of the overall health risk evaluation: (1) external and internal exposure aspects of lead; (2) lead metabolism, which determines the relationship of external lead exposure to associated health effects of lead; (3) qualitative and quantitative characterization of key health effects of lead; and (4) identification of population groups at special risk for health effects associated with lead exposure.

The various aspects of lead exposure discussed include: (1) an historical perspective on the input of lead into the environment as well as the nature and magnitude of current lead input; (2) the cycling of lead through the various environmental compartments; and (3) levels of lead in those media most relevant to lead exposure of various segments of the U.S. population. These various aspects of lead exposure are summarized in Section 13.2.

In regard to lead metabolism, some of the relevant issues addressed include: (1) the major quantitative characteristics of lead absorption, distribution, retention, and excretion in humans and how these differ between adults and children; (2) the toxicokinetic bases for external/internal lead exposure relationships with respect to both internal indicators and target tissue lead burdens; and (3) the relationships between internal and external indices of lead exposure, i.e., blood lead levels, and lead concentrations in air, food, water, and dust/soil. Section 13.3 summarizes the most salient features of lead metabolism, whereas Section 13.4 addresses experimental and epidemiological data concerning various blood lead-environmental media lead relationships.

In discussion of the various health effects of lead, the main emphasis is on the identification of those effects most relevant to various segments of the general U.S. population and the placement of such effects in a dose-effect/dose-response framework. With regard to the latter, a crucial issue has to do with relative response of various segments of the population in terms of observed effect levels as indexed by some exposure indicator. Furthermore, it is of interest to assess the extent to which available information supports the existence of a continuum of effects as one proceeds across the spectrum of exposure levels. Discussion of data on the relative number or percentage of members (i.e., "responders") of specific population groups that can be expected to experience a particular effect at various lead exposure levels is also important in order to permit delineation of dose-response curves for the relevant effects in different segments of the population. These matters are discussed in Sections 13.5 and 13.6.

Melding of information from the sections on lead exposure, metabolism, and biological effects permits the identification of population segments at special risk in terms of physiological and other host characteristics, as well as heightened vulnerability to a given effect; these risk groups are discussed in Section 13.7. With demographic identification of individuals at risk, one may then draw upon population data from other sources to obtain a numerical picture of the magnitude of population groups at potential risk. This is also discussed in Section 13.7.

Section 13.8 summarizes key information and conclusions derived from the analyses presented in the preceeding sections.

13.2 EXPOSURE ASPECTS

13.2.1 Sources of Lead Emission in the United States

The most important issues addressed here concerning the sources of lead in the human environment are: What additional pathways of human consumption have been added in the course of civilization? What are the relative contributions of natural and anthropogenic lead? From the available data, what trends can be expected in the potential consumption of lead by humans? What is the impact of normal lead cycling processes on total human exposure? And, finally, are there population segments particularly at risk due to a higher potential exposure?

Figure 13-1 is a composite of similar figures appearing in Chapters 7 and 11. This figure shows that four of the five sources of lead in the human environment are of anthropogenic origin. The only significant natural source is from the geochemical weathering of parent rock material as an input to soils. Of the four anthropogenic pathways, two are closely associated with atmospheric emissions and two (pigments and solder) are more directly related to the use of metallurgical compounds in products consumed by humans.

It is clear that natural sources contribute only a very small fraction to total lead in the biosphere. Levels of lead in the atmosphere, the main conduit for lead movement from sources into various environmental compartments, are 10,000 to 20,000-fold higher in some urban areas than in the most remote regions of the earth. Chronological records assembled

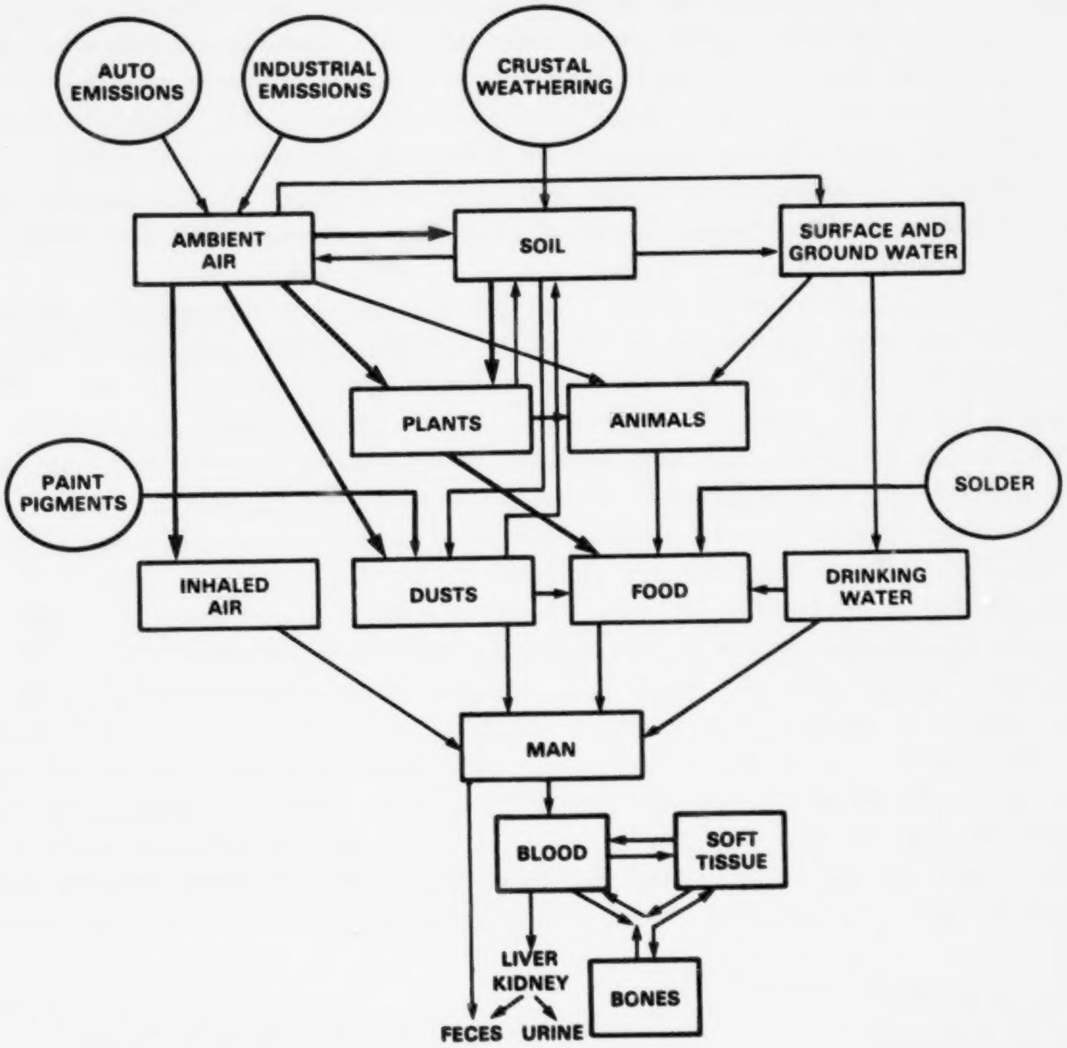
AUTO EMISSIONS
INDUSTRIAL EMISSIONS
CRUSTAL WEATHERING
AMBIENT AIR
SOIL
SURFACE AND GROUND WATER
PLANTS
ANIMALS
PAINT PIGMENTS
SOLDER
INHALED AIR
DUSTS
FOOD
DRINKING WATER
MAN
BLOOD
SOFT TISSUE
LIVER
KIDNEY
BONES
FECES
URINE

Figure 13-1. Pathways of lead from the environment to man, main compartments involved in partitioning of internal body burden of absorbed/retained lead, and main routes of lead excretion.

using reliable lead analysis techniques show that atmospheric lead levels were at least 2,000-fold lower than at present before abrupt anthropological inputs accelerated with the industrial revolution and, more recently, with the introduction of leaded gasoline. For actual comparison, estimates indicate a general background air lead level of 0.00005-0.0005 µg Pb/m^3 versus current urban air lead concentrations frequently approaching 1.0 µg Pb/m^3. A recent measurement of 0.000076 µg Pb/m^3 at the South Pole, using highly reliable lead analyses, suggests an anthropogenic enrichment factor of 13,000-fold compared to the same urban air level of 1.0 µg Pb/m^3.

Lead occupies an important niche in the U.S. economy, with consumption averaging 1.28 x 10^6 metric tons/year over the period 1971-1984. Of the various categories of lead consumption, those of pigments, gasoline additives, ammunition, foil, solder, and steel products are widely dispersed and therefore unrecoverable. In the United States, about 39,000 tons are emitted to the atmosphere each year, including 35,000 tons as gasoline additives. Lead and its compounds enter the atmosphere at various points during mining, smelting, processing, use, recycling, or disposal. Leaded gasoline combustion in vehicles accounted for 90 percent of the total anthropogenic input into the atmosphere in the United States in 1984; of the remaining 10 percent of total emissions from stationary sources, 5 percent was from the metallurgical industry, 3 percent was from waste combustion, 1 percent from combustion during energy production, and 1 percent was from miscellaneous sources. Atmospheric emissions have declined in recent years with the phase-down of lead in gasoline.

The fate of emitted particulate lead depends on particle size. It has been estimated that, of the 75 percent of combusted gasoline lead which immediately departs the vehicle in exhaust, 46 percent is in the form of particles <0.25 µm mass median aerodynamic diameter (MMAD) and 54 percent has an average particle size >10 µm. The sub-micron fraction is involved in long-range transport, whereas the larger particles settle mainly near the roadway.

13.2.2 Environmental Cycling of Lead

The atmosphere is the main conduit for movement of lead from emission sources to other environmental compartments. Removal of lead from the atmosphere occurs by both wet and dry deposition processes, each mechanism accounting for about one-half of the atmospheric lead removed. The fraction of lead emitted as alkyl lead vapor (1-6 percent) undergoes subsequent transformation to other, more stable compounds such as triethyl- or trimethyl lead as a complex function of sunlight, temperature, and ozone level.

Studies of the movement of lead emitted into the atmosphere indicate that air lead levels decrease logarithmically with distance away from the source: (1) along gradients from emission sites, e.g., roadways and smelters; (2) within urban regions away from central business districts; (3) from urban to rural areas; and (4) from developed to remote areas.

By means of wet and dry deposition, atmospheric lead is transferred to terrestrial surfaces and bodies of water. Transfer to water occurs either directly from the atmosphere or through runoff from soil to surface waters. A sizeable fraction of water-borne lead becomes lodged in aquatic sediments. Percolation of water through soil does not transport much lead to ground water because most of the lead is retained at the soil surface.

The fate of lead particles on terrestrial surfaces depends upon such factors as the mechanism of deposition, the chemical form of the particulate lead, the chemical nature of the receiving soil, and the amount of vegetation cover. Lead deposited on soils is apparently immobilized by binding to humic or fulvic acids, or by ion exchange on clays and hydrous oxides. In industrial, playground, and household environments, atmospheric particles accumulate as dusts with lead concentrations often greater than 1000 μg/g. It is important to distinguish these dusts from windblown soil dust, which typically has a lead concentration of 10-30 μg/g.

It has been estimated that soils adjacent to roadways have been enriched in lead content by as much as 10,000 μg/g soil since 1930, while in urban areas and sites adjacent to smelters as much as 130,000 μg/g has been measured in the upper 2-5 cm layer of soil.

Soil appears to be the major sink for emitted lead, with a residency half-time of decades; however, soil as a reservoir for lead cannot be considered as an infinite sink, because lead will continue to pass into the grazing and detrital food chains and sustain elevated lead levels in them until equilibrium is reached. It was estimated in Chapters 7 and 8 that soils not adjacent to major sources such as highways and smelters contain 3-5 μg/g of anthropogenic lead, and that this lead has caused an increase of lead in soil moisture by a factor of 2-4. Thus, movement of lead from soils to other environmental compartments is at least twice the prehistoric rate and will continue to increase for the foreseeable future.

Lead enters the aquatic compartment by direct transfer from the atmosphere via wet and dry deposition, as well as indirectly from the terrestrial compartment via surface runoff. Water-borne lead, in turn, may be retained in some soluble fraction or may undergo sedimentation, depending on such factors as pH, temperature, suspended matter which may entrap lead, etc. Present levels of lead in natural waters represent a 50-fold enrichment over background content, from 0.02 to 1.0 μg/l, due to anthropogenic activity. Surface waters receiving urban effluent represent a 2500-fold and higher enrichment (50 μg Pb/l and higher).

13.2.3 Levels of Lead in Various Media of Relevance to Human Exposure

Human populations in the United States are exposed to lead in air, food, water, and dust. In rural areas, Americans not occupationally exposed to lead are estimated to consume 40-60 μg Pb/day. This level of exposure is referred to as the baseline exposure for the American population because it is unavoidable except by drastic change in lifestyle or by regulation of lead in foods or ambient air. There are several environmental circumstances that can increase human exposures above baseline levels. Most of these circumstances involve the accumulation of atmospheric dusts in the work and play environments. A few, such as pica and family home gardening, may involve consumption of lead in chips of exterior or interior house paint.

13.2.3.1 Ambient Air Lead Levels. Monitored ambient air lead concentration values in the United States are contained in two principal data bases: (1) EPA's National Air Sampling Network (NASN), recently renamed National Filter Analysis Network (NFAN); and (2) EPA's National Aerometric Data Bank, consisting of measurements by state and local agencies in conjunction with compliance monitoring for the current ambient air lead standard.

NASN data for 1982, the latest year in the annual surveys for which valid distinctions can be made between urban and non-urban stations, indicate that most urban sites show reported annual averages below 0.7 μg Pb/m^3, while the majority of non-urban locations have annual figures below 0.2 μg Pb/m^3. Over the interval 1976-1984, there has been a downward trend in these averages, mainly attributable to decreasing lead content of leaded gasoline and the increasing usage of lead-free gasoline. Furthermore, examination of quarterly averages over this interval shows a typical seasonal variation, characterized by maximum air lead values in summer and minimum values in winter.

With respect to the particle size distribution of ambient air lead, EPA studies using cascade impactors in six U.S. cities have indicated that 60-75 percent of such air lead was associated with sub-micron particles. This size distribution is significant in considering the distance particles may be transported and the deposition of particles in the pulmonary compartment of the respiratory tract. The relationship between airborne lead at the monitoring station and the lead inhaled by humans is complicated by such variables as vertical gradients, relative positions of the source, the monitor, and the person, and the ratio of indoor to outdoor lead concentrations. Personal monitors would probably be the most effective means to obtain an accurate picture of the amount of lead inhaled during the normal activities of an individual. However, the information gained would be insignificant, considering that inhaled lead is generally only a small fraction of the total lead exposure, compared to the lead in food, beverages, and dust. The critical question in regard to airborne lead is how much lead becomes entrained in dust. In this respect, the existing monitoring network may provide an adequate estimate of the air concentration from which the rate of deposition can be determined.

13.2.3.2 Levels of Lead In Dust. The lead content of dusts can figure prominently in the total lead exposure picture for young children. Lead in aerosol particles deposited on rigid surfaces in urban areas (such as sidewalks, porches, steps, parking lots, etc.) does not undergo dilution compared to lead transferred by deposition onto soils. Lead in dust can approach extremely high concentrations and can accumulate in the interiors of dwellings as well as in the outside surroundings, particularly in urban areas.

Measurements of soil lead to a depth of 5 cm in areas of the United States were shown in one study to range from 150 to 500 µg/g dry weight close to roadways (i.e., within 8 meters). By contrast, lead in dusts deposited on or near heavily traveled traffic arteries show levels in major U.S. cities ranging up to 8000 µg/g and higher. In residential areas, exterior dust lead levels are approximately 1000 µg/g or less if contaminated only by atmospheric lead. Levels of lead in house dust can be significantly elevated; a study of house dust samples in Boston and New York City revealed levels of 1000-2000 µg/g. Some soils adjacent to houses with exterior lead-based paints may have lead concentrations greater than 10,000 µg/g.

Forty-four percent of the baseline consumption of lead by children is estimated to result from consumption of 0.1 g of dust per day (Tables 13-1 and 13-2). Ninety percent of this dust lead is of atmospheric origin. Dust also accounts for more than 90 percent of the additive lead attributable to living in an urban environment or near a smelter (Table 13-3).

13.2.3.3 Levels of Lead in Food. The route by which adults and older children in the baseline population of the United States receive the largest proportion of lead intake is through foods, with reported estimates of the dietary lead intake for Americans ranging from 35 to 55 µg/day. The added exposure from living in an urban environment is about 28 µg/day for adults and 91 µg/day for children, all of which can be attributed to atmospheric lead.

Atmospheric lead may be added to food crops in the field or pasture, during transportation to the market, during processing, and during kitchen preparation. Metallic lead, mainly solder, may be added during processing and packaging. Other sources of lead, as yet undetermined, increase the lead content of food between the field and dinner table. American children, adult females, and adult males consume 21, 32, and 45 µg Pb/day, respectively, in food and beverages. Of these amounts, 45-65 percent is of direct atmospheric origin, 25-37 percent is of metallic origin, and 5-8 percent is of undetermined origin.

Processing of foods, particularly canning, can significantly add to their background lead content, although it appears that the impact of this is being lessened with the trend away from use of lead-soldered cans. The canning process can increase lead levels 8-to 10-fold higher than for the corresponding uncanned food items. Home food preparation can also be a source of additional lead in cases where food preparation surfaces are exposed to moderate amounts of high-lead household dust.

13.2.3.4 Lead Levels in Drinking Water. Lead in drinking water may result from contamination of the water source or from the use of lead materials in the water distribution system. Lead entry into drinking water from the latter is increased in water supplies which are plumbosolvent, i.e., with a pH below 6.5. Exposure of individuals occurs through direct ingestion of the water or via food preparation in such water.

TABLE 13-1. SUMMARY OF BASELINE HUMAN EXPOSURES TO LEAD
(μg/day)

		Soil				
Source	Total lead consumed	Natural lead consumed	Indirect atmospheric lead*	Direct atmospheric lead*	Lead from solder or other metals	Lead of undetermined origin
Child-2 yr old						
Inhaled air	0.5	0.001	-	0.5	-	-
Food, Water & beverages	25.1	0.71	1.7	10.3	11.2	1.2
Dust	21.0	0.6	-	19.0	-	1.4
Total	46.6	1.3	1.7	29.8	11.2	2.6
Percent	100%	2.8%	3.5%	64.0%	24.0%	5.6%
Adult female						
Inhaled air	1.0	0.002	-	1.0	-	-
Food, Water & beverages	32.0	0.91	2.4	12.6	8.2	1.5
Dust	4.5	0.2	-	2.9	-	1.4
Total	37.5	1.2	2.5	17.4	13.5	2.9
Percent	100%	3.1%	6.6%	46.5%	36.1%	7.8%
Adult male						
Inhaled air	1.0	0.002	-	1.0	-	-
Food, Water & beverages	45.2	1.42	3.5	19.3	18.9	2.2
Dust	4.5	0.2	-	2.9	-	1.4
Total	50.7	1.6	3.5	23.2	18.9	3.6
Percent	100%	3.1%	6.8%	45.8%	37.2%	7.0%

*Indirect atmospheric lead has been previously incorporated into soil, and will probably remain in the soil for decades or longer. Direct atmospheric lead has been deposited on the surfaces of vegetation and living areas or incorporated during food processing prior to human consumption.

Source: This report.

TABLE 13-2. RELATIVE BASELINE HUMAN LEAD EXPOSURES EXPRESSED PER KILOGRAM BODY WEIGHT*

	Total lead consumed, µg/day	Total lead consumed per kg body wt, µg/kg·day	Atmospheric lead per kg body wt, µg/kg·day
Child (2-yr-old)			
Inhaled air	0.5	0.05	0.05
Food and beverages	25.1	2.5	1.0
Dust	21.0	2.1	1.9
Total	46.6	4.65	2.95
Adult female			
Inhaled air	1.0	0.02	0.02
Food and beverages	32.0	0.64	0.25
Dust	4.5	0.09	0.06
Total	37.5	0.75	0.33
Adult male			
Inhaled air	1.0	0.014	0.014
Food and beverages	45.2	0.65	0.28
Dust	4.5	0.064	0.04
Total	50.7	0.73	0.334

*Body weights: 2-year-old child = 10 kg; adult female = 50 kg; adult male = 70 kg.

Source: This report.

The major source of lead contamination of drinking water is the distribution system itself, particularly in older urban areas. Highest levels are encountered in "first-draw" samples, i.e., water sitting in the piping system for an extended period of time. In a large community water supply survey of 969 systems carried out in 1969-1970, it was found that the prevalence of samples exceeding 0.05 µg/g was greater where water was plumbo-solvent.

Most drinking water, and the beverages produced from drinking water, contain 0.007-0.011 µg Pb/g. The exceptions are canned juices and soda pop, which range from 0.018 to 0.040 µg/g. About 15 percent of the lead consumed in drinking water and beverages is of direct atmospheric origin; 60 percent comes from solder and other metals.

TABLE 13-3. SUMMARY OF POTENTIAL ADDITIVE EXPOSURES TO LEAD (μg/day)

	Total lead consumed, μg/day	Atmospheric lead consumed, μg/day	Other lead sources, μg/day
Baseline exposure:			
Child			
Inhaled air	0.5	0.5	-
Food, water & beverages	25.1	10.3	14.8
Dust	21.0	19.0	2.0
Total baseline	46.6	29.8	16.8
Additional exposure due to:			
Urban atmospheres[1]	91	91	
Family gardens[2]	48	12	36
Interior lead paint[3]	110		110
Residence near smelter[4]	880	880	
Secondary occupational[5]	150		
Baseline exposure:			
Adult male			
Inhaled air	1.0	1.0	-
Food, water & beverages	54.7	20.3	34.4
Dust	4.5	2.9	1.6
Total baseline	60.2	24.2	36.0
Additional exposure due to:			
Urban atmospheres[1]	28	28	
Family gardens[2]	120	30	17
Interior lead paint[3]	17		
Residence near smelter[4]	100	100	
Occupational[6]	1100	1100	
Secondary occupational[5]	44		
Smoking[7]	30	27	3
Wine consumption[8]	100	?	?

[1] Includes lead from household (1000 μg/g) and street dust (1500 μg/g) and inhaled air (0.75 μg/m^3).

[2] Assumes soil lead concentration of 2000 μg/g; all fresh leafy and root vegetables, sweet corn of Table 7-12 replaced by produce from garden. Also assumes 25% of soil lead is of atmospheric origin.

[3] Assumes household dust rises from 300 to 2000 μg/g. Dust consumption remains the same as baseline.

[4] Assumes household and street dust increases to 10,000 μg/g.

[5] Assumes household dust increases to 2400 μg/g.

[6] Assumes 8-hr shift at 10 μg Pb/m^3 or 90% efficiency of respirators at 100 μg Pb/m^3, and occupational dusts at 100,000 μg/m^3.

[7] One-and-a-half packs per day.

[8] Assumes unusually high consumption of one liter per day.

Source: This report.

13.2.3.5 Lead in Other Media. Flaking lead paint as well as paint chips and weathered powdered paint in and around deteriorated housing stock in urban areas of the Northeast and Midwest has long been recognized as a major source of lead exposure for young children residing in this housing stock, particularly for children with pica. Census data, for example, indicate that there are approximately 27 million residential units in the United States built before 1940, many of which still contain lead-based paint. Also, individuals who are cigarette smokers may inhale significant amounts of lead in tobacco smoke. One study has indicated that the smoking of 30 cigarettes daily results in lead intake equivalent to that of inhaling lead in ambient air at a level of 1.0 $\mu g/m^3$.

13.2.3.6 Cumulative Human Lead Intake From Various Sources. Table 13-1 shows the baseline of human lead exposures in the United States as described in detail in Chapter 7. These data show that atmospheric lead accounts for at least 45 percent of the baseline adult consumption and 60 percent of the daily consumption by a 2-yr-old child. These percentages are conservative estimates because a part of the lead of undetermined origin may originate from atmospheric lead not yet accounted for.

From Table 13-2, it can be seen that young children have a dietary lead intake rate that is 5-fold greater than for adults, on a body weight basis. To these observations must be added that absorption rates for lead are higher in children than in adults by at least 3-fold. Overall, then, the rate of lead entry into the blood stream of children, on a body weight basis, is estimated to be twice that of adults from the respiratory tract and six to nine times greater from the GI tract. Since children consume more dust than adults, the atmospheric fraction of the baseline exposure is sixfold higher for children than for adults, on a body weight basis. These differences generally tend to place young children at greater risk, in terms of relative amounts of atmospheric lead absorbed per kg body weight, than adults under any given lead exposure situation.

13.3 LEAD METABOLISM: KEY ISSUES FOR HUMAN HEALTH RISK EVALUATION

From the detailed discussion of those various quantifiable characteristics of lead toxicokinetics in humans and animals presented in Chapter 10, several clear issues emerge as being important for full evaluation of the human health risk posed by lead:

(1) Differences in systemic or internal lead exposure of groups within the general population in terms of such factors as age/development and nutritional status; and

(2) The relationship of indices of internal lead exposures to both environmental levels of lead and tissues levels/effects.

Item 1 is used along with additional information on relative sensitivity to lead health effects to provide the basis for identifying segments within human populations at increased risk in terms of exposure criteria. Item 2 deals with the adequacy of current means for assessing internal lead exposure in terms of providing adequate margins of protection from lead exposures which produce health effects of concern.

13.3.1 Differential Internal Lead Exposure Within Population Groups

Compared to adults, young children take in more lead through the gastrointestinal and respiratory tracts on a unit body weight basis, absorb a greater fraction of this lead intake, and also retain a greater proportion of the absorbed amount. Unfortunately, such amplification of these basic toxicokinetic parameters in children versus adults also occurs at the time when: (1) humans are developmentally more vulnerable to the effects of toxicants such as lead in terms of metabolic activity; and (2) the interactive relationships of lead with such factors as nutritive elements are such as to induce a negative course toward further exposure risk.

Typical of physiological differences in children versus adults in terms of lead exposure implications is a more metabolically active skeletal system in children. In children, turnover rates of bone elements such as calcium and phosphorus are greater than in adults, with correspondingly greater mobility of bone-sequestered lead. This activity is a factor in the observation that the skeletal system of children is relatively less effective as a depository for lead than in adults.

Metabolic demand for nutrients, particularly calcium, iron, phosphorus, and the trace elements is such that widespread deficiencies of these nutrients exist, particularly among poor children. The interactive relationships of all of these elements with lead are such that deficiency states enhance lead absorption and/or retention. In the case of lead-induced reductions in 1,25-dihydroxyvitamin D, furthermore, there may exist an increasingly adverse interactive cycle between lead effects on 1,25-dihydroxyvitamin D and associated increased absorption of lead.

Quite apart from the physiological differences which enhance internal lead exposure in children is the unique relationship of 2- to 3-year-olds to their exposure setting by way of normal mouthing behavior and the extreme manifestation of this behavior, pica. This behavior occurs in the same age group which studies have consistently identified as having a peak in blood lead levels. A number of investigations have addressed the quantification of this particular route of lead exposure, and it is by now clear that such exposure will dominate other routes when the child's surroundings, e.g., dust and soil, are significantly contaminated by lead.

Information provided in Chapter 10 also makes it clear that lead traverses the human placental barrier, with lead uptake by the fetus occurring throughout gestation. Such uptake of lead poses a potential threat to the fetus via an impact on the embryological development of the central nervous and other systems. Hence, the only logical means of protecting the fetus from lead exposure is exposure control during pregnancy. Within the general population, then, young children and pregnant women qualify as well-defined groups at high risk for lead exposure.

In addition, certain emerging information (noted in Section 13.5 and described in detail in the Addendum to this document) indicates that increases in blood pressure are associated with blood lead concentrations ranging from ≥30-40 μg/dl down to possibly as low as 7 μg/dl; this association appears to be particularly robust in white males, aged 40-59. Occupational exposure to lead, particularly among lead workers, logically defines these individuals as also being in a high-risk category; work place contact is augmented by those same routes and levels of lead exposure affecting the rest of the adult population. From a biological point of view, lead workers do not differ from the general adult population with respect to the various toxicokinetic parameters and any differences in exposure control--occupational versus non-occupational populations--as they exist are based on factors other than toxicokinetics.

13.3.2 Indices of Internal Lead Exposure and Their Relationship To External Lead Levels and Tissue Burdens/Effects

Several points are of importance to consider in the area of lead toxicokinetics: (1) the temporal characteristics of indices of lead exposure; (2) the relationship of these indicators to external lead levels; (3) the validity of indicators of exposure in reflecting target tissue burdens; (4) the interplay between these indicators and lead in body compartments; and (5) those various aspects of this issue that, in particular, refer to children.

At this time, blood lead is widely held to be the most convenient, if imperfect, index of both lead exposure and relative risk for various adverse health effects. In terms of exposure, however, it is generally accepted that blood lead is a temporally variable measure which yields an index of relatively recent exposure because of the rather rapid clearance of absorbed lead from the blood. Such a measure, then, is of limited usefulness in cases where exposure is variable or intermittent over time, as is often the case with pediatric lead exposure. Mineralizing tissue (specifically deciduous teeth), on the other hand, accumulate lead over time in proportion to the degree of lead exposure, and analysis of this material provides an assessment integrated over a greater time period.

These two methods of assessing internal lead exposure have obvious shortcomings. A blood lead value will say little about any excessive lead intake at early periods, even though such remote exposure may have resulted in significant injury. On the other hand, whole tooth or dentine analysis is retrospective in nature and can only be done after the particularly vulnerable age in children--under 4-5 years--has passed. Such a measure, then, provides little utility upon which to implement regulatory policy or clinical intervention.

It may be possible to resolve the dilemmas posed by these existing methods by in situ analysis of teeth and bone lead, such that the intrinsic advantage of mineral tissue as a cumulative index is combined with measurement which is temporally concordant with on-going exposure. Work in several laboratories offers promise for such in situ analysis (See Chapters 9 and 10).

A second issue concerning internal indices of exposure to environmental lead is the relationship of changes in lead content of some medium with changes in blood content. Much of Chapter 11 was given over to description of the mathematical relationships of blood lead with lead in some external medium--air, food, water, etc.--without consideration of the biological underpinnings for these relationships.

Over a relatively broad range of lead exposure through some medium, the relationship of lead in the external medium to lead in blood is curvilinear, such that relative change in blood lead per unit change in medium level generally becomes increasingly less as exposure increases. This behavior may reflect changes in tissue lead kinetics, reduced lead absorption, or increased excretion. With respect to changes in body lead distribution, the relative amount of whole blood lead in plasma increases significantly with increasing whole blood lead content; i.e., the plasma/erythrocyte ratio increases. Limited animal data would suggest that changes in absorption may be one factor in this phenomenon. In any event, modest changes in blood lead levels with exposure at the higher end of this range are in no way to be taken as reflecting concomitantly modest changes in body or tissue lead uptake. Evidence continues to accumulate which suggests that an indicator such as blood lead is an imperfect measure of tissue lead burdens and of changes in such tissue levels in relation to changes in external exposure (see Figure 13-2).

In Chapter 10, it was pointed out that blood lead is logarithmically related to chelatable lead (the latter being a more useful measure of the potentially toxic fraction of body lead), such that a unit change in blood lead is associated with an increasingly larger amount of chelatable lead. One consequence of this relationship is that moderately elevated blood lead values will tend to mask the "margin of safety" in terms of mobile body lead burdens. Such masking is apparent in several studies where chelatable lead levels in children showing moderate elevations in blood lead overlapped those obtained in subjects showing frank plumbism, i.e., overt lead intoxication. In a multi-institutional survey involving several hundred children, it was found that a significant percentage of children with moderately elevated blood lead values had chelatable lead burdens which qualified them for medical treatment.

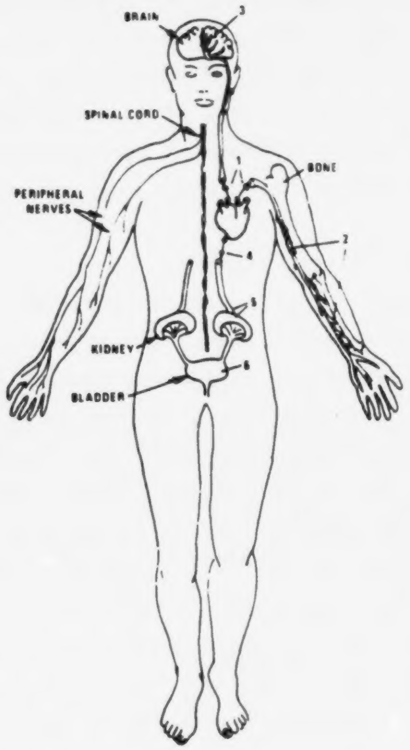

Figure 13-2. Illustration of main body compartments involved in partitioning, retention, and excretion of absorbed lead and selected target organs for lead toxicity. Inhaled and ingested lead circulates via blood (1) to mineralizing tissues such as teeth and bone (2), where long-term retention occurs reflective of cumulative past exposures. Concentrations of lead in blood circulating to "soft tissue" target organs such as brain (3), peripheral nerve, and kidney, reflect both recent external exposures and lead recirculated from internal reservoirs (e.g. bone). Blood lead levels used to index internal body lead burden tend to be in equilibrium with lead concentrations in soft tissues and, below 30 μg/dl, also generally appear to reflect accumulated lead stores. However, somewhat more elevated current blood lead levels may "mask" potentially more toxic elevations of retained lead due to relatively rapid declines in blood lead in response to decreased external exposure. Thus, provocative chelation of some children with blood leads of 30-40 μg/dl, for example, results in mobilization of lead from bone and other tissues into blood and movement of the lead (4) into kidney (5), where it is filtered into urine and excreted (6) at concentrations more typical of overtly lead-intoxicated children with higher blood lead concentrations.

Related to the above is the question of the source of chelatable lead. It was noted in Chapter 10 that some sizable fraction of chelatable lead is derived from bone and that this compels reappraisal of the notion that bone is an "inert sink" for otherwise toxic body lead. The notion of bone lead as toxicologically inert never did accord with what was known from studies of bone physiology, i.e., that bone is a "living" organ. The thrust of recent studies of chelatable lead, as well as interrelationships of lead and bone metabolism, supports the view that bone lead is actually an insidious source of long-term systemic lead exposure rather than a protective mechanism which permits significant lead contact in industrialized populations.

The complex interrelationships of lead exposure, blood lead, and lead in body compartments is of particular interest in considering the disposition of lead in young children. Since children take in more lead on a weight basis, and absorb and retain more of this lead than the adult, one might expect that either tissue and blood levels would be significantly elevated or that the child's skeletal system would be more efficient in lead sequestration. Average blood lead levels in young children are generally either similar to adult males or somewhat higher than for adult females. Limited autopsy data, furthermore, indicate that soft tissue levels in children are not markedly different from adults, whereas the skeletal system shows an approximate 2-fold increase in lead concentration from infancy to adolescence. Neglected in this observation is the fact that the skeletal system in children grows at an exponential rate, so that skeletal mass increases 40-fold during the interval in childhood when bone lead levels increase 2-fold; this results in an actual increase of approximately 80-fold in total skeletal lead. If the skeletal growth factor is taken into account, along with growth in soft tissue and the expansion of vascular fluid volumes, the question of lead disposition in children is better understood. Finally, limited animal data indicate that blood lead alterations with changes in lead exposure are poor indicators of such changes in target tissue. Specifically, it appears that abrupt reduction of lead exposure will be more rapidly reflected by decreases in blood lead than by decreased lead concentrations in such target tissues as the central nervous system, especially in the developing organism. This discordance may underlie the observation that severe lead neurotoxicity in children is associated with a rather broad range of blood lead values (see Section 12.4).

The above discussion of some of the problems with the use of blood lead in assessing target tissue burdens or the toxicologically active fraction of total body lead is really a summary of the toxicokinetic problems inherent with use of blood lead levels in defining margins of safety for avoiding internal exposure or undue risk of adverse effects. If, for example, blood lead levels of 30-50 μg/dl in "asymptomatic" children are associated with chelatable lead burdens which overlap those encountered in frank pediatric plumbism, as documented in several studies of lead-exposed children, then there is no margin of safety at these blood levels for severe effects which are not at all a matter of controversy. Were it both logistically feasible to do so on a large scale and were the use of chelants free of health risk to the subjects, serial provocative chelation testing would appear to be the better indicator of exposure and risk. Failing this, the only prudent alternative is the use of a large safety factor applied to blood lead which would translate to an "acceptable" chelatable burden. It is likely that this blood lead value would lie well below the currently accepted upper limit of 25 µg/dl (U.S. Centers for Disease Control, 1985), since the safety factor would have to be large enough to protect against frank plumbism as well as more subtle health effects seen with non-overt lead intoxication. This rationale from the standpoint of lead toxicokinetics is also in accord with the growing data base for dose-response relationships of lead's effects on heme biosynthesis, erythropoiesis, and the nervous system in humans as detailed in Sections 12.3 and 12.4 (see also Section 13.5, below).

Further development and routine use of *in situ* mineral tissue testing at time points concordant with on-going exposure and the comparison of such results with simultaneous blood lead and chelatable lead measurement would be of significant value in further defining what level of blood lead is indeed an acceptable upper limit.

13.4 DEMOGRAPHIC CORRELATES OF HUMAN LEAD EXPOSURE AND RELATIONSHIPS BETWEEN EXTERNAL AND INTERNAL LEAD EXPOSURE INDICES

13.4.1 Demographic Correlates of Lead Exposure

Studies of ancient populations using bone and teeth show that levels of internal exposure of lead today are substantially elevated over past levels. Studies of current populations living in remote areas far from urbanized cultures show blood lead levels in the range of 1-5 µg/dl. In contrast to the blood lead levels found in remote populations, data from current U.S. populations generally have geometric means ranging from 10 to 20 µg/dl depending on age, race, sex, and degree of urbanization. These higher blood lead levels in the United States appear to be associated with industrialization and widespread commercial use of lead, e.g., in gasoline combustion.

Age appears to be one of the most important demographic covariates of blood lead levels. Blood lead levels in children up to six years of age are generally higher than those in non-occupationally exposed adults. Children aged two to three years tend to have the highest levels, as shown in Figure 13-3. Blood lead levels in non-occupationally exposed adults may increase slightly with age due to skeletal lead accumulation.

Sex has a differential impact on blood lead levels, depending on age. No significant differences exist between males and females less than seven years of age; males above the age of seven generally have higher blood lead levels than females. Race also plays a role, in

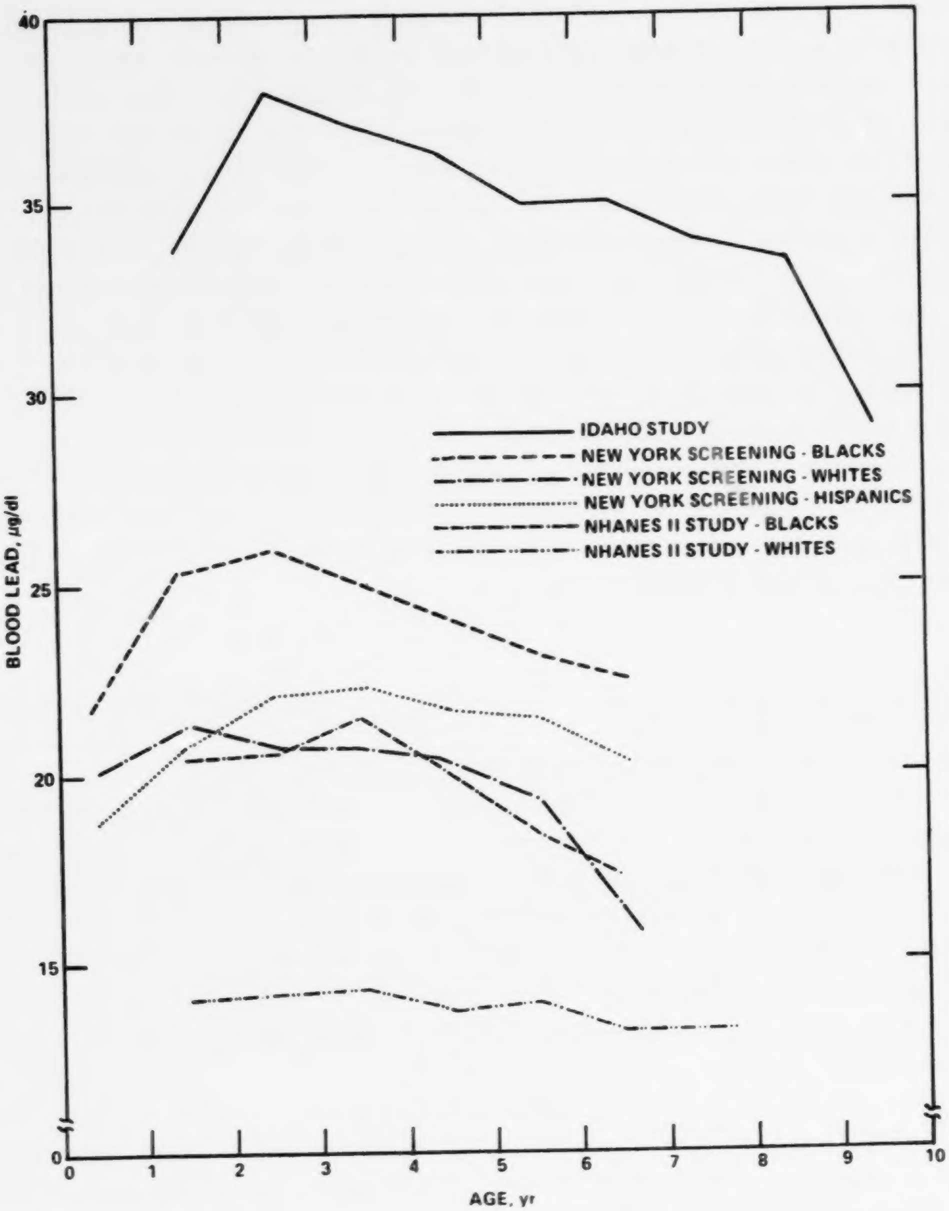

Figure 13-3. Geometric mean blood lead levels by race and age for younger children in the NHANES II Study (Annest et al., 1982), the Kellogg Silver Valley, Idaho Study (Yankel et al., 1977), and the New York Childhood Screening Studies (Billick et al., 1979).

that blacks have higher blood lead levels on average than either whites or Hispanics. The reason for this is not yet fully understood; genetic factors have yet to be fully disentangled from differential exposure circumstances and other factors.

Blood lead levels also seem to increase with degree of urbanization. Data from NHANES II show that blood lead levels in the United States, averaged from 1976 to 1980, increase from a geometric mean of 11.9 μg/dl in rural populations to 12.8 μg/dl in urban populations less than one million, and increase again to 14.0 μg/dl in urban populations of one million or more.

Recent U.S. blood lead levels show a downward trend occurring consistently across race, age, and geographic location. This pattern commenced in the early part of the 1970's and has continued into 1980. The downward trend has occurred from a shift in the entire distribution and not through a truncation in the high blood lead levels. This consistency suggests a general causative factor, and attempts have been made to identify the causative element. Reduction in lead emitted from the combustion of leaded gasoline is a prime candidate (See discussion under 13.4.2).

Distribution of blood lead levels, examined on a population basis, generally have similarly skewed distributions. That is, blood lead levels from populations thought to be homogenous in terms of demographic and lead exposure characteristics generally follow an approximately lognormal distribution. Geometric standard deviations (an estimation of dispersion) for observed distributions from four different studies discussed in Chapter 11 (including analytic error) are about 1.4 for children and possibly somewhat smaller for adults. This allows an estimation of the upper tail of the blood lead distribution for the U.S. population, which would be the population segment expected to be at greater risk.

13.4.2 Relationships Between External and Internal Lead Exposure Indices

There is no question that, across a broad spectrum of external air lead concentrations ranging upward to beyond 10-20 μg/m^3, the relationship between air lead exposures and increases in blood lead levels is nonlinear. However, because one main purpose of this document is to examine relationships of lead in air and lead in blood under ambient conditions, the results of studies most appropriate for this purpose were emphasized in Chapter 11. A summary of the most appropriate studies appears in Table 13-4. At air lead exposures of 3.2 μg/m^3 or less, there is no statistically significant difference between curvilinear and linear blood lead inhalation relationships. Also, for air lead exposures of 10 μg/m^3 or more, either nonlinear or linear relationships can be fitted. Thus, a reasonably consistent picture emerges in which the blood lead-air lead relationship by direct inhalation appears to be approximately linear in the range of normal ambient exposures (0.1-2.0 μg/m^3) as discussed in Chapter 7. Differences among individuals in a given study, and among several studies are large, so that

TABLE 13-4. SUMMARY OF BLOOD INHALATION SLOPES (β)

Population	Study	Study Type	N	Slope (β), μg/dl per μg/m^3	Model sensitivity of slope[a]
Children	Angle and McIntire (1979), Omaha, NE	Population	1074	1.92	(1.40 - 4.40)[b,c,d]
	Roels et al. (1980), Belgium	Population	148	2.46	(1.55 - 2.46)[b,c]
	Yankel et al. (1977); Walter et al. (1980), Idaho	Population	879	1.52	(1.07 - 1.52)[b,c,d]
Adult Males	Azar et al. (1975), five groups	Population	149	1.32	(1.08 - 2.39)[c,d]
	Griffin et al. (1975), NY prisoners	Experiment	43	1.75	(1.52 - 3.38)[e]
	Gross (1979)	Experiment	6	1.25	(1.25 - 1.55)[c]
	Rabinowitz et al. (1973,1976, 1977)	Experiment	5	2.14	(2.14 - 3.51)[f]

[a] Selected from among the most plausible statistically equivalent models. For nonlinear models, slope at 1.0 μg/m^3.

[b] Sensitive to choice of other correlated predictors such as dust and soil lead.

[c] Sensitive to linear vs. nonlinear at low air lead.

[d] Sensitive to age as a covariate.

[e] Sensitive to baseline changes in controls.

[f] Sensitive to assumed air lead exposure.

pooled estimates of the blood lead inhalation slope depend upon the the weight given to various studies. Several studies were selected for analysis, based upon factors described earlier. EPA analyses of experimental and clinical studies (Griffin et al., 1975; Rabinowitz et al., 1973, 1976, 1977; Kehoe 1961a,b,c; Gross 1981; Hammond et al., 1981) suggest that blood blood lead in adults increases by approximately 1.64 ± 0.22 µg/dl from direct inhalation of each additional µg/m^3 of air lead. EPA anaylsis of Azar's population study (Azar et al., 1975) yields a slope of 1.32 ± 0.38 for adult males. EPA analyses of other population studies (Yankel et al., 1977; Roels et al., 1980; Angle and McIntire, 1979) suggest that, for children, the blood lead increase is approximately 1.92 (a median estimate) µg/dl per each µg/m^3 increment of air lead.

These slope estimates are based on the assumption that an equilibrium level of blood lead is achieved within a few months after exposure begins. This is only approximately true, since lead stored in the skeleton may return to blood after some years. Chamberlain et al. (1978) suggest that long-term inhalation slopes should be about 30 percent larger than these estimates. Inhalation slopes quoted here are associated with a half-life of blood lead in adults of about 30 days. O'Flaherty et al. (1982) suggest that the blood lead half-life may increase slightly with duration of exposure, but this has not been confirmed (Kang et al., 1983).

One possible approach would be to regard all inhalation slope studies as equally informative and to calculate an average slope using reciprocal squared standard error estimates as weights. This approach has been rejected for two reasons. First, the standard error estimates characterize only the internal precision of an estimated slope, not its representativeness (i.e., bias) or predictive validity. Secondly, experimental and clinical studies obtain more information from a single individual than do population studies. Thus, it may not be appropriate to combine the two types of studies.

While estimates of the inhalation slope for children are only available from population studies, the importance of dust ingestion as a non-inhalation pathway for children is established by many studies. Slope estimates have been derived for air lead inhalation based on several such studies (e.g., Angle and McIntire 1979; Roels et al., 1980; Yankel et al., 1977) from which the air inhalation and dust ingestion contributions can both be estimated. Brunekreef (1984) reviewed these and other studies and found wide variations in slope estimates that include aggregate impacts of direct inhalation and indirect dust contributions. Such aggregate analyses from some of the better conducted studies yield slope estimates in the general range of 4-6 µg/dl blood lead per µg/m^3 air lead increase. Also, results from another recent analysis (Angle et al., 1984) suggest that indirect soil/dust contributions contribute blood lead increases of 4-5 µg/dl in addition to the direct inhalation contribution of 1.92 µg/dl blood lead per µg/m^3 air lead.

While direct inhalation of air lead is stressed, this is not the only air lead contribution that needs to be considered. Smelter studies allow partial assessment of the air lead contributions to soil, dust, and finger lead. Conceptual models allow preliminary estimation of the propagation of lead through the total food chain as shown in Chapter 7; useful mathematical models to quantify this propagation through the food chain need to be developed. The direct inhalation relationship does provide useful information on changes in blood lead in response to changes in air lead on a time scale of several months. However, the indirect pathways through dust and soil and through the food chain may delay the total blood lead response to changes in air lead, perhaps by one or more years. The Italian ILE study facilitates partial assessment of this delayed response from leaded gasoline as a source.

Dietary absorption of lead varies greatly from one person to another and depends on the physical and chemical form of the carrier, on nutritional status, and on whether lead is ingested with food or between meals. These distinctions are particularly important for consumption by children of leaded paint, dust, and soil. Typical values of 10 percent absorption of ingested lead into blood have been assumed for adults and 25-50 percent for children.

It is difficult to determine accurate relationships between blood lead levels and lead levels in food or water. While dietary intake must be estimated by duplicate diets or fecal lead determinations, water lead levels can be determined with some accuracy. However, the varying amounts of water consumed by different individuals add to the uncertainty of the estimated relationships.

Quantitative analyses relating blood lead levels and dietary lead exposures have been reported. While studies on infants provide estimates that are in close agreement, only one individual study is available for adults (Sherlock et al. 1982); another estimate from a number of pooled studies is also available. These two estimates are in good agreement. Most of the subjects in the Sherlock et al. (1982) and United Kingdom Central Directorate on Environmental Pollution (1982) studies received quite high dietary lead levels (>300 μg/day). The fitted cube root equations give high slopes at lower dietary lead levels. On the other hand, the linear slope of the United Kingdom Central Directorate on Environmental Pollution (1982) study is probably an underestimate of the slope at lower dietary lead levels. For these reasons, the Ryu et al. (1983) study appears to be the most reliable, although it only applies to infants. Estimates for adults can be obtained from the experimental studies, but would most appropriately apply at high exposure levels (e.g., >300 μg/day). In such studies, most of the dietary lead intake supplements were so high that many of the subjects had blood lead concentrations much in excess of 30 μg/dl for a considerable part of the experiment. The blood lead levels thus may not have completely reflected lead exposure, due to the previously noted nonlinearity of blood lead response at high exposures. The slope estimates for adult dietary intake are about 0.02 μg/dl increase in blood lead per μg lead/day total intake, but consideration of blood lead kinetics may increase this value to about 0.04. Such values are a bit lower than those estimated from the population studies extrapolated to typical dietary intakes: that is, about 0.05 µg/dl per µg/day. The Ryu et al. (1983) value for infants is much larger, being about 0.16 µg/dl per µg/day.

The relation between blood lead and water lead is not clearly defined and is often described as nonlinear. Water lead intake varies greatly from one person to another. It has been assumed that children can absorb 25-50 percent of lead in water. Some authors chose to fit cube root models to their data, although polynomial and logarithmic models were also used. Unfortunately, the form of the model greatly influences the estimated contributions to blood lead levels from relatively low water lead concentrations.

Although there is close agreement in the quantitative analyses of the relationship between blood lead level and dietary lead, there is a larger degree of variability in results of the various water lead studies. Over a wide range of water lead concentrations, the relationship is curvilinear, but its exact form has yet to be determined. At typical ambient water levels for U.S. populations, the relationship appears linear. The only study that determines the relationship based on lower water lead values (<100 µg/l) is the Pocock et al. (1983) study. The data from this study, as well as the authors themselves, suggest that the relationship is linear in this lower range of water lead levels. Furthermore, the estimated contributions to blood lead levels in this study are quite consistent with the polynomial models from other studies. For these reasons, the Pocock et al. (1983) slope of 0.06 is considered to represent the best estimate. The possibility still exists, however, that the higher estimates of the other studies may be correct in certain situations, especially at higher water lead levels (>100 µg/l).

Studies relating soil lead to blood lead levels are difficult to compare. The relationship obviously depends on depth of soil lead, sampling method, cleanliness of the home, age of the children, mouthing activities of the children, and possibly many other factors. Various soil sampling methods and sampling depths have been used over time, and as such they may not be directly comparable and may produce a dilution effect of the major lead concentration contribution from dust, which is located primarily in the top 2 cm of the soil. Increases in soil dust lead significantly increase blood lead in children. From several studies (Yankel et al., 1977; Angle and McIntire, 1979) EPA estimates an increase of 0.6-6.8 µg/dl in blood lead for each increase of 1000 µg/g in soil lead concentration. The values from the Stark et al. (1982) study of about 2 µg/dl per 1000 µg/g may represent a reasonable median estimate. The relationship of housedust lead to blood lead is difficult to obtain. Household dust also increases blood lead, as children from the cleanest homes in the Silver Valley/Kellogg Study had 6 µg/dl less lead in blood, on average, than those from the households with the most dust.

A number of specific environmental sources of airborne lead have been evaluated for potential direct influence on blood lead levels. Combustion of leaded gasoline appears to be an extremely important contributor to airborne lead in the United States, as indicated by strong associations between reductions in nationwide gasoline lead usage and average U.S. blood lead levels determined by a major population survey on a nationwide level. Studies of data from blood lead screening programs in specific U.S. metropolitan areas also suggest that the downward trend in blood lead levels noted earlier is due to reductions in air lead levels, mainly attributable to reductions of lead in gasoline.

In addition, other studies used isotope ratios of lead to estimate the relative proportion of lead in the blood coming from airborne lead or, more specifically, from leaded gasoline usuage. For example, from the Isotopic Lead Experiment (ILE) data of Facchetti and Geiss (1982) and Facchetti (1985), as shown in Table 13-5, the direct inhalation of air lead may account for 60 percent of the total adult blood lead uptake from leaded gasoline in a large urban center, but inhalation is a much less important pathway in suburban parts of the region (19 percent of the total gasoline lead contribution) and in the rural parts of the region (9 percent of the total gasoline lead contribution). EPA analyses of the preliminary results from the ILE study separated the inhalation and non-inhalation contributions of leaded gasoline to blood lead into the following three parts: (1) an increase of about 1.7 µg/dl in blood lead per µg/m^3 of air lead, attributable to direct inhalation of the combustion products of leaded gasoline; (2) a sex difference of about 2 µg/dl attributable to lower exposure of women to indirect (non-inhalation) pathways for gasoline lead; and (3) a non-inhalation background attributable to indirect gasoline lead pathways, such as ingestion of dust and food, increasing from about 2 µg/dl in Turin to 3 µg/dl in remote rural areas. The non-inhalation background represents only two to three years of environmental accumulation at the new experimental lead isotope ratio. It is not clear how to numerically extrapolate these estimates to subpopulations in the United States; however, it is evident that even in rural and suburban parts of a metropolitan area, the indirect (non-inhalation) pathways for exposure to leaded gasoline make a significant contribution to blood lead. This can be seen in Table 13-5. It should also be noted that the blood lead isotope ratio responded fairly rapidly when the gasoline lead isotope ratio returned to its pre-experimental value, but it is not yet possible to estimate the long-term change in blood lead attributable to persistent exposures to accumulated environmental lead.

The strongest kind of scientific evidence about causal relationships is based on an experiment in which all possible extraneous factors are controlled. The evidence derived from the Isotopic Lead Experiment comes very close. The experimental intervention consisted of

TABLE 13-5. ESTIMATED CONTRIBUTION OF LEADED GASOLINE TO BLOOD LEAD BY INHALATION AND NON-INHALATION PATHWAYS

Location	Air lead fraction from gasoline[a]	Mean air lead conc.,[b] μg/m^3	Blood Pb fraction from gasoline[c]	Mean blood lead conc.,[d] μg/dl	Blood Pb from gasoline,[e] μg/dl	Pb from gasoline in air,[f] μg/dl	Non-inhaled Pb from gasoline,[g] μg/dl	Estimated fraction gas-lead inhalation[h]
Turin	0.873	2.0	0.214	21.77	4.66	2.79	1.80	0.60
<25 km	0.587	0.56	0.114	25.06	2.86	0.53	2.33	0.19
>25 km	0.587	0.30	0.101	31.78	3.21	0.28	2.93	0.09

[a] Fraction of air lead in Phase 2 attributable to lead in gasoline.

[b] Mean air lead in Phase 2, μg/m^3.

[c] Mean fraction of blood lead in Phase 2 attributable to lead in gasoline.

[d] Mean blood lead concentration in Phase 2, μg/dl.

[e] Estimated blood lead from gasoline = (c) x (d).

[f] Estimated blood lead from gas inhalation = β x (a) x (b), $\beta = 1.6$.

[g] Estimated blood lead from gas, non-inhalation = (f)-(e).

[h] Fraction of blood lead uptake from gasoline attributable to direct inhalation = (f)/(e).

Data: Facchetti and Geiss (1982); Facchetti (1985).

replacing the normal ^{206}Pb/^{207}Pb isotope ratio by a very different ratio. There is no plausible mechanism by which other concurrent lead exposure variables (food, water, beverages, paint, industrial emissions) could have also changed their isotope ratios. Hence the very large changes in isotope ratios in blood were responding to the change in gasoline. Our analyses (Chapter 11) show that consideration of inhalation of community air lead alone probably substantially underestimates the total effect of gasoline lead, at least in the 35 subjects whose blood leads were tracked in the ILE Preliminary Study. Spengler et al. (1984), as discussed in Section 11.3, also suggest that part of the extra exposure could possibly be attributed to exposure to higher-than-ambient air lead concentrations inside motor vehicles, e.g., on the trip to work; however, no data are presently available to confirm this hypothesis.

Primary lead smelters, secondary lead smelters, and battery plants emit lead directly into the air and ultimately increase soil and dust lead concentrations in their vicinity. Adults, and especially children, have been shown to exhibit elevated blood lead levels when living close to these sources. Blood lead levels in these residents have been shown to be related to air lead, as well as to soil or dust lead exposures. In addition, individuals (especially children) living in housing units with deteriorating or weathering lead-based paint may also be exposed via lead accumulated in dust or soils within or around their dwellings.

13.4.3 Proportional Contributions of Lead in Various Media to Blood Lead in Human Populations

The various mathematical descriptions of the relationship of blood lead to lead in individual media--air, food, water, dust, soil--were discussed in some detail in Chapter 11 and concisely in the preceding section (13.4.2) of this chapter. Using values for lead intake/content of these media which appear to represent the current exposure picture for human populations in the United States, these relationships are further employed in this section to estimate proportional inputs to total blood lead levels in U.S. children. Such an exercise is of help in providing an overall perspective on which routes of exposure are of most significance in terms of contributions to blood lead levels seen especially in urban children, the population group in the United States at greatest risk for lead exposure and its toxic effects.

Table 13-6 tabulates the relative direct contributions of air lead to blood lead at different air lead levels for calculated typical background levels of lead from food, water, and dust for children in the United States. Also listed are the direct and indirect contributions of air lead to blood lead at varying air lead levels for children, given calculated typical background levels of blood lead. Calculations and assumptions used in deriving the estimates shown in Table 13-6 are summarized in footnotes to that table. The diet contributions listed in the table, for example, are based on the following: (1) estimated average background levels of lead (from non-air and air sources) in food ingested per day by children, as delineated in Table 7-19; and (2) the value of 0.16 µg/dl of blood increase per µg/day food lead intake found by Ryu et al. (1983) for infants. Similarly, values for other parameters used in Table 13-6 are obtained from work discussed in Chapters 7 and 11.

It is of interest to compare (1) estimated blood lead values predicted in Table 13-6 to occur at particular air lead concentrations with (2) actual blood lead levels observed for children living in the United States in areas with comparable ambient air concentrations. As an example, NHANES II survey results for children living in rural areas and urban areas of more than one million population or less than one million were presented in Table 11-5. For children (aged 0.5-5 yr) living in urban areas of >1 million, the mean blood lead value was 16.8 µg/dl, a value representative of average blood lead levels nationwide for preschool children living in large urban areas during the NHANES survey period (1976 to February, 1980).

Ambient air lead concentrations (quarterly averages) during the same time period (1976-1979) for a geographically diverse sample of large urban areas in the United States (population >1 million) are available from Table 7-3. The air lead levels during 1976-1979 averaged 1.06 μg/m³ for all cities listed in Table 7-3 and 1.12 μg/m³ for eight cities in the table that were included in the NHANES II study (i.e., Boston, New York, Philadelphia, Detroit, Chicago, Houston, Los Angeles, and Washington, DC). The Table 13-6 blood lead values of 12.6-14.6 μg/dl estimated for air lead levels of 1.0-1.25 μg/m³ approximate the observed NHANES II average of 16.8 μg/dl for children in large urban areas with average air lead levels of 1.06-1.12 μg/m³. The NHANES II blood lead values for preschool children would be expected to be somewhat higher than the estimates in Table 13-6 because the latter were derived from FDA data for 1981-1983, which were lower than the FDA values for the 1976-1980 NHANES II period (see Chapter 7). FDA data for food, water, and beverages for the 1976-1980 period are not in a form exactly comparable to the 1981-1983 data used in calculating background contributions in Table 13-6, but do suggest that lead levels in those media declined by about 20 percent from the 1976-1980 period to 1981-1983. If background contributions in Table 13-6 were corrected (i.e., increased by 20 percent) to be comparable to the 1976-1980 period, then the blood lead levels of children exposed to 1.25 μg/m³ air lead would increase to 15.5 μg/dl, a value even closer to the mean of 16.8 μg/dl found for NHANES II children living in urban environments (>1 million) during 1976-1980.

13.5 BIOLOGICAL EFFECTS OF LEAD RELEVANT TO THE GENERAL HUMAN POPULATION

13.5.1 Introduction

It is clear from the wealth of available literature reviewed in Chapter 12 that there exists a continuum of biological effects associated with lead across a broad range of exposure. At rather low levels of lead exposure, biochemical changes, e.g., disruption of certain enzymatic activities involved in heme biosynthesis and erythropoietic pyrimidine metabolism, are detectable. Heme biosynthesis is a generalized process in mammalian species, including man, with importance for normal physiological functioning of virtually all organ systems. With increasing lead exposure, there are sequentially more intense effects on heme synthesis as well as a broadening of effects to additional biochemical and physiological mechanisms in various tissues. In addition to heme biosynthesis impairment at relatively low levels of lead exposure, disruption of normal functioning of the erythropoietic and nervous systems are among the earliest effects observed as a function of increasing lead exposure. With increasingly intense exposure, more severe disruption of the erythropoietic and nervous systems occur and additional organ systems are affected, resulting, for example, in manifestation of renal

TABLE 13-6. CONTRIBUTIONS FROM VARIOUS MEDIA TO BLOOD LEAD LEVELS (µg/dl) OF U.S. CHILDREN (AGE = 2 YEARS): BACKGROUND LEVELS AND INCREMENTAL CONTRIBUTIONS FROM AIR

Source	Air lead, µg/m³						
	0	0.25	0.50	0.75	1.00	1.25	1.50
Background-non air							
Food, Water and Beverages[a]	2.37	2.37	2.37	2.37	2.37	2.37	2.37
Dust[b]	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Subtotal	2.67	2.67	2.67	2.67	2.67	2.67	2.67
Background-air							
Food, Water and Beverages[c]	1.65	1.65	1.65	1.65	1.65	1.65	1.65
Ingested dust (with Pb deposited from air)[d]	0.00	1.57	3.09	4.70	6.27	7.84	9.40
Inhaled air[e]	0.00	0.50	1.00	1.50	2.00	2.50	3.00
Total	4.32	6.39	8.41	10.52	12.59	14.66	16.72

[a] From Table 7-19, (25.1 - 10.3) µg/day x (0.16 from Ryu et al., 1983) = 2.37 µg/dl.

[b] From Chapter 7, 1/10 dust not atmospheric. Using Angle et al. (1984) low area (Area S) for soil and house dust and their regression equation, we have: (1/10) x (97 µg/g x 0.00681 + 324 µg/g x 0.00718) = 0.30 µg/dl. Alternatively, the consumption from non air would be (1/10) x (97 µg/g soil dust + 324 µg/g house dust) x 0.05 grams ingested of each = 2.1 µg ingested. Using Ryu et al. (1983), 2.1 x 0.16 = 0.34 µg/dl added to blood.

[c] As in (a) above, but using 10.3 instead of (25.1 - 10.3) yields 1.67 µg/dl. Values are derived for component of background Pb in food from past deposition from air onto soil and into other media leading into human food chain (not expected to change much except over long-term).

[d] The regression equations of Angle et al. (1984) are used, as well as levels of soil dust and house dust in the low area (S) and high area (C) of that study. For example, the increase at 1.0 µg/m³ in air would result in increases in soil as follows:

$$\frac{1.00 - 0.29}{0.86 - 0.29} \times (519 - 97) = 526\ \mu g/g$$

Similarly the increase in house dust would be:

$$\frac{1.00 - 0.29}{0.86 - 0.29} \times (625 - 324) = 374\ \mu g/g$$

The effect on blood lead would be (526 x 0.00681) + (374 x 0.00718) = 6.27 µg/dl.

[e] Using the 2.0 slope from Angle et al. (1984), i.e., 1.93 rounded up.

effects, disruption of reproductive functions, and impairment of immunological functions. At sufficiently high levels of exposure, the damage to the nervous system and other effects can be severe enough to result in death or, in some cases of non-fatal lead poisoning, long-lasting sequelae such as permanent mental retardation.

As discussed in Chapter 12 of this document, numerous new studies, reviews, and critiques concerning lead-related health effects have been published since the issuance of the earlier EPA Lead Criteria Document in 1977. Of particular importance for present criteria development purposes are those new findings, taken together with information available at the writing of the 1977 Lead Criteria Document, which have bearing on the establishment of quantitative dose-effect or dose-response relationships which can be potentially viewed as adverse health effects likely to occur among the general population at or near existing ambient air concentrations of lead in the United States. Key information regarding observed health effects and their implications are discussed below for adults and children.

For the latter group, children, emphasis is placed on the discussion of (1) heme biosynthesis effects, (2) certain other biochemical and hematological effects, and (3) the disruption of nervous system functions. All of these appear to be among those effects of most concern for potential occurrence in association with exposure to existing U.S. ambient air lead levels for the population group at greatest risk for lead-induced health effects (i.e., children ≤6 years old). Emphasis is also placed on the delineation of internal lead exposure levels, as defined mainly by blood lead (PbB) levels likely associated with the occurrence of such effects. Also discussed are characteristics of the subject effects that are of crucial importance with regard to the determination of which might reasonably be viewed as constituting "adverse health effects" in affected human populations.

Over the years, there have been superimposed on the continuum of lead-induced biological effects various judgments as to which specific effects observed in man constitute "adverse health effects." Such judgments involve not only medical consensus regarding the health significance of particular effects and their clinical management, but also incorporate societal value judgments. Such societal value judgments often vary depending upon the specific overall contexts in which they are applied; e.g., in judging permissible exposure levels for occupational versus general population exposures to lead. For some lead exposure effects, e.g., severe nervous system damage resulting in death or serious medical sequelae consequent to intense lead exposure, there exists little or no disagreement as to these being significant "adverse health effects." For many other effects detectable at sequentially lower levels of lead exposure, however, the demarcation lines as to which effects represent adverse health effects and the lead exposure levels at which they are accepted as occurring are neither sharp nor fixed, having changed markedly during the past several decades. That is, from an historical perspective, levels of lead exposure deemed to be acceptable for either occupationally-exposed persons or the general population have been steadily revised downward as more sophisticated biomedical techniques have revealed formerly unrecognized biological effects and concern has increased in regard to the medical and social significance of such effects. As a concrete example, pediatric blood lead concentrations deemed to be associated with unacceptable risk of lead toxicity have been repeatedly revised downward by the U.S. Public Health Service (see CDC, 1985).

It is difficult to provide a definitive statement of all criteria by which specific biological effects associated with any given agent can be judged to be "adverse health effects." Nevertheless, several criteria are currently well-accepted as helping to define which effects should be viewed as "adverse." These include the following: (1) impaired normal functioning of a specific tissue or organ system itself; (2) reduced reserve capacity of that tissue or organ system in dealing with stress due to other causative agents; (3) the reversibility/irreversibility of the particular effect(s); (4) the relative frequency of a given effect; (5) presence of the effect in a vulnerable segment of the population; and (6) the cumulative or aggregate impact of various effects on individual organ systems on the overall functioning and well-being of the individual.

Examples of possible uses of such criteria in evaluating lead effects can be cited for illustrative purposes. For example, impairment of heme synthesis intensifies with increasing lead exposure until hemoprotein synthesis is inhibited in many organ systems, leading to reductions in such functions as oxygen transport, cellular energetics, neurotransmitter functions, detoxification of xenobiotic agents, and biosynthesis of important substances such as 1,25-dihydroxyvitamin D. In Figure 13-4, the far-ranging impact of lead on the body heme pool and associated disruption of many physiological processes is depicted, based on data discussed in Sections 12.2 and 12.3. Furthermore, inspection of Figure 13-4 reveals effects that can be viewed as intrinsically adverse as well as those that reduce the body's ability to cope with other forms of toxic stress, e.g., reduced hepatic detoxification of many types of xenobiotics and, possibly, impairment of the immune system. The liver effect can also be cited as an example of reduced reserve capacity pertinent to consideration of the effects of lead, as the reduced capacity of the liver to detoxify certain drugs or other xenobiotic agents results from lead effects on hepatic detoxification enzyme systems.

In regard to the issue of reversibility/irreversibility of lead effects, there are really two dimensions to the issue that need to be considered, i.e.: (1) biological reversibility or irreversibility characteristic of the particular effect in a given organism; and (2) the generally less-recognized concept of exposure reversibility or irreversibility. Severe central

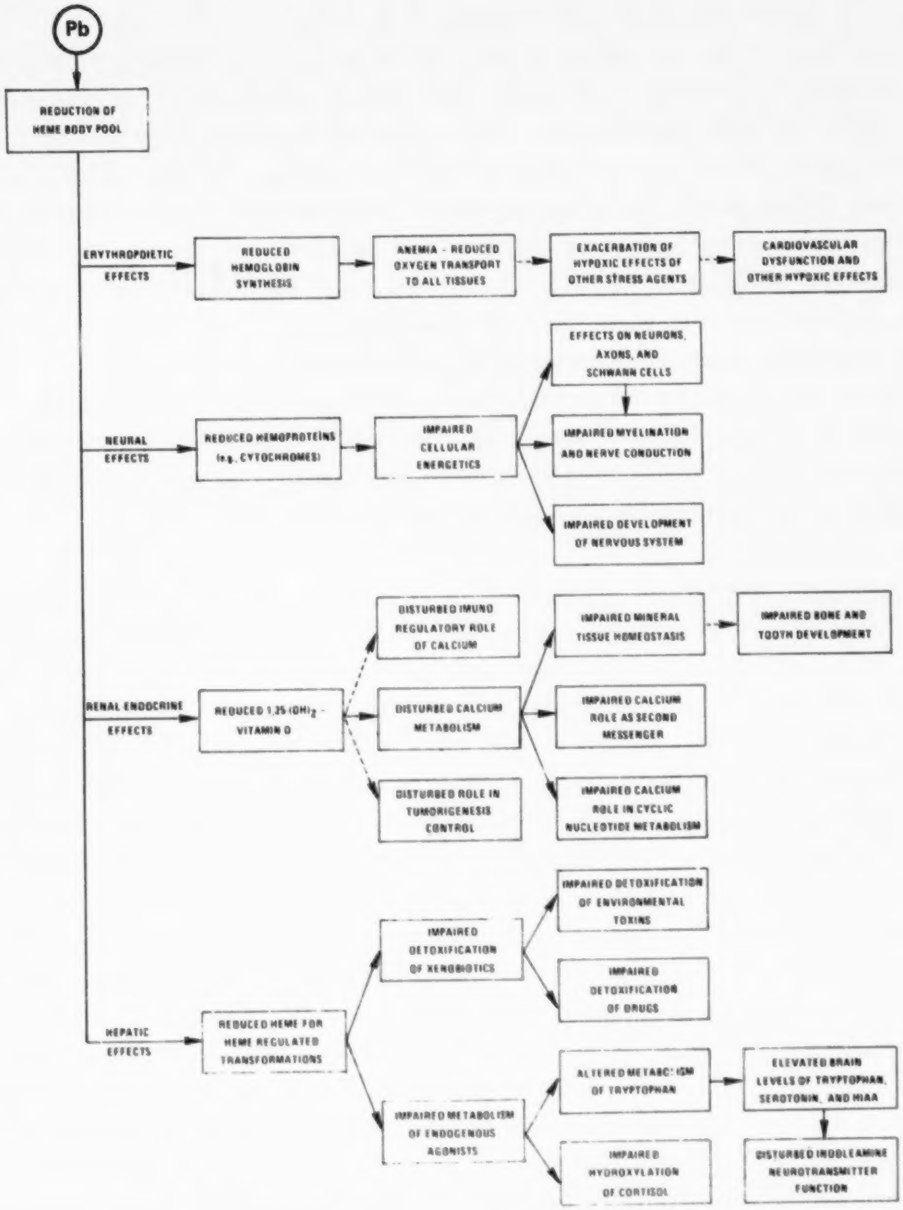
Pb
REDUCTION OF HEME BODY POOL
ERYTHROPOIETIC EFFECTS
REDUCED HEMOGLOBIN SYNTHESIS
ANEMIA - REDUCED OXYGEN TRANSPORT TO ALL TISSUES
EXACERBATION OF HYPOXIC EFFECTS OF OTHER STRESS AGENTS
CARDIOVASCULAR DYSFUNCTION AND OTHER HYPOXIC EFFECTS
NEURAL EFFECTS
REDUCED HEMOPROTEINS (e.g., CYTOCHROMES)
IMPAIRED CELLULAR ENERGETICS
EFFECTS ON NEURONS, AXONS, AND SCHWANN CELLS
IMPAIRED MYELINATION AND NERVE CONDUCTION
IMPAIRED DEVELOPMENT OF NERVOUS SYSTEM
RENAL ENDOCRINE EFFECTS
REDUCED 1,25 (OH)$_2$ - VITAMIN D
DISTURBED IMUNO REGULATORY ROLE OF CALCIUM
DISTURBED CALCIUM METABOLISM
DISTURBED ROLE IN TUMORIGENESIS CONTROL
IMPAIRED MINERAL TISSUE HOMEOSTASIS
IMPAIRED BONE AND TOOTH DEVELOPMENT
IMPAIRED CALCIUM ROLE AS SECOND MESSENGER
IMPAIRED CALCIUM ROLE IN CYCLIC NUCLEOTIDE METABOLISM
HEPATIC EFFECTS
REDUCED HEME FOR HEME REGULATED TRANSFORMATIONS
IMPAIRED DETOXIFICATION OF XENOBIOTICS
IMPAIRED DETOXIFICATION OF ENVIRONMENTAL TOXINS
IMPAIRED DETOXIFICATION OF DRUGS
IMPAIRED METABOLISM OF ENDOGENOUS AGONISTS
ALTERED METABOLISM OF TRYPTOPHAN
ELEVATED BRAIN LEVELS OF TRYPTOPHAN, SEROTONIN, AND HIAA
DISTURBED INDOLEAMINE NEUROTRANSMITTER FUNCTION
IMPAIRED HYDROXYLATION OF CORTISOL

Figure 13-4. Multi-organ impact of reductions of heme body pool by lead. Impairment of heme synthesis by lead (see Section 12.3) results in disruption of a wide variety of important physiological processes in many organs and tissues. Particularly well documented are erythropoietic, neural, renal-endocrine, and hepatic effects indicated above by solid arrows (——►). Plausible further consequences of heme synthesis interference by lead which remain to be more conclusively established are indicated by dashed arrows (- - -►).

nervous system damage resulting from intense, high-level lead exposure is generally accepted as an irreversible effect of lead exposure; the reversibility/irreversibility of certain more difficult-to-detect neurological effects occurring at lower lead exposure levels, however, remains a matter of some controversy. The concept of exposure reversibility/irreversibility can be illustrated by the case of urban children of low socioecomomic status showing disturbances in heme biosynthesis and erythropoiesis. Biologically, these various effects may be considered reversible; the extent to which actual reversibility occurs, however, is determined by the feasibility of removing these subjects from their particular lead exposure setting. If such removal from exposure is unlikely or does not occur, then such effects will logically persist and, defacto, constitute essentially irreversible effects.

The issues of frequency of effects and vulnerable segments of the population in whom these effects occur are intimately related. As detailed later in Section 13.7, young children--particularly inner-city children--constitute a high risk group because they do show a high frequency of certain health effects, as summarized below.

13.5.2 Dose-Effect Relationships for Lead-Induced Health Effects

13.5.2.1 Human Adults. The lowest observed effect levels (in terms of blood lead concentrations) thus far credibly associated with particular health effects of concern for human adults in relation to specific organ systems or generalized physiological processes, e.g., heme synthesis, are summarized in Table 13-7. That table should be viewed as representing lowest blood lead levels thus far credibly associated with unacceptable risk for a given effect occurring among at least some adults. As such, many other individuals may not experience the stated effect until distinctly higher blood lead levels are reached, due to wide ranges of individual biological susceptibility, variations in nutritional status, and other factors.

The most serious effects associated with markedly elevated blood lead levels are severe neurotoxic effects that include irreversible brain damage, as indexed by the occurrence of acute or chronic encephalopathic symptoms observed in both humans and experimental animals. For most human adults, such damage typically does not occur until blood lead levels exceed 100-120 μg/dl. Often associated with encephalopathic symptoms at these or higher blood lead levels are severe gastrointestinal symptoms and objective signs of effects on several other organ systems. Precise threshold(s) for occurrence of overt neurological and gastrointestinal signs and symptoms of lead exposure in cases of subencephalopathic lead intoxication remain to be established, but such effects have been observed in adult lead workers at blood lead levels as low as 40-60 μg/dl, notably lower than levels earlier thought to be "safe" for adult lead exposure. Other types of health effects occur coincident with the above overt neurological and gastrointestinal symptoms indicative of marked lead intoxication. These range from frank peripheral neuropathies to chronic nephropathy and anemia.

TABLE 13-7. SUMMARY OF LOWEST OBSERVED EFFECT LEVELS FOR KEY LEAD-INDUCED HEALTH EFFECTS IN ADULTS

Lowest observed effect level (PbB)*	Heme synthesis and hematological effects	Neurological effects	Effects on the kidney	Reproductive function effects	Cardiovascular effects
100-120 µg/dl		Encephalopathic signs and symptoms	Chronic nephropathy		
80 µg/dl	Frank anemia				
60 µg/dl				Female reproductive effects	
50 µg/dl	Reduced hemoglobin production	Overt subencephalopathic neurological symptoms		Altered testicular function	
40 µg/dl	Increased urinary ALA and elevated coproporphyrins	Peripheral nerve dysfunction (slowed nerve conduction)			
30 µg/dl					Elevated blood pressure (White males,) aged 40-59
25-30 µg/dl	Erythrocyte protoporphyrin (EP) elevation in males				
15-20 µg/dl	Erythrocyte protoporphyrin (EP) elevation in females				
<10 µg/dl	ALA-D inhibition				?

*PbB = blood lead concentrations.

Source: This report.

Toward the lower range of blood lead levels associated with overt lead intoxication or somewhat below, less severe but important signs of impairment in normal physiological functioning in several organ systems are evident among apparently asymptomatic lead-exposed adults, including the following: (1) slowed nerve conduction velocities indicative of peripheral nerve dysfunction (at levels as low as 30-40 µg/dl); (2) altered testicular function (at 40-50 µg/dl); and (3) reduced hemoglobin production (at approximately 50 µg/dl) and other signs of impaired heme synthesis evident at still lower blood lead levels. All of these effects point toward a generalized impairment of normal physiological functioning across several different organ systems, which becomes abundantly evident as adult blood lead levels exceed 30-40 µg/dl. Evidence for impaired heme synthesis effects in blood cells exists at still lower blood lead levels in adults, as does evidence for elevated blood pressure in middle-aged white males (aged 40-59). The significance of impaired heme synthesis effects and evidence of impairment of other biochemical processes important in cellular energetics are discussed below in relation to children.

13.5.2.2 Children. Table 13-8 summarizes lowest observed effect levels for a variety of important health effects observed in children. Again, as for adults, it can be seen that lead impacts many different organ systems and biochemical/physiological processes across a wide range of exposure levels. Also, again, the most serious of these effects is the severe, irreversible central nervous system damage manifested in terms of encephalopathic signs and symptoms. In children, effective blood lead levels for producing encephalopathy or death are lower than for adults, starting at approximately 80-100 µg/dl. Permanent severe mental retardation and other marked neurological deficits are among lasting neurological sequelae typically seen in cases of non-fatal childhood lead encephalopathy. Other overt neurological signs and symptoms of subencephalopathic lead intoxication are evident in children at lower blood lead levels (e.g., peripheral neuropathies detected in some children at levels as low as 40-60 µg/dl). Chronic nephropathy, indexed by aminoaciduria, is most evident at high exposure levels over 100 µg/dl, but may also exist at lower levels (e.g., 70-80 µg/dl). In addition, colic and other overt gastrointestinal symptoms clearly occur at similar or still lower blood lead levels in children, at least down to 60 µg/dl. Frank anemia is also evident by 70 µg/dl, representing an extreme manifestation of the reduced hemoglobin synthesis observed at blood lead levels as low as 40 µg/dl, along with other signs of marked inhibition of heme synthesis at that exposure level. All of these effects are reflective of the widespread marked impact of lead on the normal physiological functioning of many different organ systems and some are evident in children at blood lead levels as low as 40 µg/dl; and all of them are widely accepted as being clearly adverse health effects.

Additional studies demonstrate evidence for further, important health effects occurring in non-overtly lead-intoxicated children at similar or lower blood lead levels than those

TABLE 13-8. SUMMARY OF LOWEST OBSERVED EFFECT LEVELS FOR KEY LEAD-INDUCED HEALTH EFFECTS IN CHILDREN

Lowest observed effect level (PbB)*	Heme synthesis and hematological effects	Neurological effects	Renal system effects	Gastrointestinal effects
80-100 µg/dl		Encephalopathic signs and symptoms	Chronic nephropathy (aminoaciduria, etc.)	Colic, other overt gastrointestinal symptoms ↓
70 µg/dl	Frank anemia			---
60 µg/dl		Peripheral neuropathies ↓		
50 µg/dl		?		
40 µg/dl	Reduced hemoglobin synthesis	Peripheral nerve dysfunction (slowed NCV's)		
	Elevated coproporphyrin	CNS cognitive effects (IQ deficits, etc.)		
	Increased urinary ALA	|		
30 µg/dl		? |	Vitamin D metabolism interference	
15 µg/dl	Erythrocyte protoporphyin elevation	Altered CNS electrophysiological responses	↓	
10 µg/dl	ALA-D inhibition |	↓ ?	?	
	Py-5-N† activity inhibition ↓ ?			

*PbB = blood lead concentrations.

†Py-5-N = pyrimidine-5'-nucleotidase.

Source: This report.

indicated above for overt intoxication effects. Among the most important of the effects discussed in Chapter 12 are neuropsychological and electrophysiological effects evaluated as being associated with low-level lead exposures in non-overtly lead-intoxicated children. Indications of peripheral nerve dysfunction, indexed by slowed nerve conduction velocities (NCV), have been shown in children down to blood lead levels as low as 30 µg/dl. As for CNS effects, none of the available studies on the subject, individually, can be said to prove conclusively that significant cognitive (IQ) or behavioral effects occur in children at blood lead levels <30 µg/dl. However, the most recent neurobehavioral studies of CNS cognitive (IQ) effects collectively demonstrate associations between neuropsychologic deficits and low-level lead exposures in young children resulting in blood lead levels ranging to below 30 µg/dl. The magnitudes of average observed IQ deficits generally appear to be approxiamtely 5 points at mean blood lead levels of 50-70 µg/dl, about 4 points at mean blood lead levels of 30-50 µg/dl, and 1-2 points at mean blood lead levels of 15-30 µg/dl. Somewhat larger decrements have been reported for the latter blood lead range among children of lower socioeconomic status families.

Additional recent studies have obtained results at blood lead values mainly in the 15-30 µg/dl range indicative of small, but not unimportant, effects of lead on the ability to focus attention, appropriate social behavior, and other types of behavioral performance. However, due to specific methodological problems with each of these various studies (as noted in Chapter 12), much caution is warranted that precludes conclusive acceptance of the observed effects being due to lead rather than other (at times uncontrolled for) potentially confounding variables. This caution is particularly warranted in view of other well-conducted studies that have appeared in the literature which did not find statistically significant associations between lead and similar effects at blood lead levels below 30 µg/dl. Still, because such latter studies even found some small effects remaining after correction for confounding factors, lead cannot be ruled out as an etiological factor contributing to the induction of such effects in the 15-30 µg/dl range, based on existing published studies.

Also of considerable importance are studies which provide evidence of changes in EEG brain wave patterns and CNS evoked potential responses in non-overtly lead intoxicated children. The work of Burchfiel et al. (1980) indicates significant associations between IQ decrements, EEG pattern changes, and lead exposures among children with average blood lead levels falling in the range of 30-50 µg/dl. Research results provided by Otto et al. (1981, 1982, 1983) also demonstrate clear, statistically significant associations between electrophysiological (SW voltage) changes and blood lead levels in the range of 30-55 µg/dl and analogous associations at blood lead levels below 30 µg/dl (with no evident threshold down to 15 µg/dl or somewhat lower). In this case, the presence of electrophysiological changes observed upon follow-up of some of the same children two years and five years later suggests persistence of such effects even in the face of later declines in blood lead levels and, therefore, possible long-term persistence of the observed electrophysiological CNS changes. However, the reported electrophysiological effects in this case were not found to be significantly associated with IQ decrements.

While the precise medical or health significance of the neuropsychological and electrophysiological effects found by the above studies to be associated with low-level lead exposures is difficult to fully define at this time, the IQ deficits and other behavioral changes likely impact the intellectual development, school performance, and social development of the affected children sufficiently so as to be regarded as adverse. This is especially true if such impaired intellectual development or school performance and disrupted social development are reflective of persisting, long-term effects of low-level lead exposure in early childhood. The issue of persistence of such lead effects still remains to be more clearly resolved, with some study results reviewed in Chapter 12 and mentioned above suggesting relatively short-lived or markedly decreasing lead effects on neuropsychological functions over a few years from early to later childhood and other studies suggesting that significant low-level lead-induced neurobehavioral and EEG effects may, in fact, persist into later childhood. Despite any remaining ambiguities of the above type, however, the medical community has highlighted (CDC, 1985) lead-induced neurobehavioral effects (e.g., IQ deficits and other neuropsychologic effects) as one basis for viewing pediatric blood lead levels below 25-30 µg/dl as being associated with unacceptable risk for lead-induced toxicity.

In regard to additional studies reviewed in Chapter 12 concerning the neurotoxicity of lead, certain evidence exists which suggests that neurotoxic effects may be associated with lead-induced alterations in heme synthesis, resulting in an accumulation of ALA in brain which affects CNS GABA synthesis, binding, and/or inactivation by neuronal reuptake after synaptic release. Also, available experimental data suggest that these effects may have functional significance in the terms of this constituting one mechanism by which lead may increase the sensitivity of rats to drug-induced seizures and, possibly, by which GABA-related behavioral or physiological control functions are disrupted. Unfortunately, the available research data do not allow credible direct estimates of blood lead levels at which such effects might occur in rats, other non-human mammalian species, or man. Inferentially, however, one can state that threshold levels for any marked lead-induced ALA impact on CNS GABA mechanisms are most probably at least as high as blood lead levels at which significant accumulations of ALA have been detected in erythrocytes or non-blood soft tissues (see below). Regardless of any dose-effect levels inferred, though, the functional and/or medical significance of lead-induced ALA effects on CNS mechanisms at low levels of lead exposure remains to be more fully determined and cannot, at this time, be unequivocably seen as an adverse health effect.

Research concerning lead-induced effects on heme synthesis also provides information of importance in evaluating what blood lead levels are associated with significant health effects in children. As discussed earlier, in Chapter 12 and Section 13.4, lead affects heme synthesis at several points in its metabolic pathway, with consequent impact on the normal functioning of many body tissues. The activity of the enzyme ALA-S, catalyzing the rate-limiting step of heme synthesis, does not appear to be significantly affected until blood lead levels reach or exceed approximately 40 μg/dl. The enzyme ALA-D, which catalizes the conversion of ALA to porphobilinogen as a further step in the heme biosynthetic pathway, appears to be affected at much lower blood lead levels as indexed directly by observations of ALA-D inhibition or indirectly in terms of consequent accumulations of ALA in blood and non-blood tissues. More specifically, inhibition of erythrocyte ALA-D activity has been observed in humans and other mammalian species at blood lead levels even below 10-15 μg/dl, with no clear threshold evident. Correlations between erythrocyte and hepatic ALA-D activity inhibition in lead workers at blood lead levels in the range of 12-56 μg/dl suggest that ALA-D activity in soft tissues (e.g., brain, liver, kidney, etc.) may be inhibited at similar blood lead levels at which erythrocyte ALA-D activity inhibition occurs, resulting in accumulations of ALA in both blood and soft tissues.

Some studies indicate that increases in both blood and urinary ALA occur below the current commonly-accepted blood lead level of 40 μg/dl. Such increases in blood and urinary ALA are detectable in humans at blood lead levels below 30 μg/dl, with no clear threshold evident down to 15-20 μg/dl, although other data exist which fail to show any relationship below 40 μg/dl blood lead. Other studies have demonstrated significant elevations in rat brain, spleen, and kidney ALA levels consequent to acute or chronic lead exposure, but no clear blood lead levels can yet be specified at which such non-blood tissue ALA increases occur in humans. It is reasonable to assume, however, that ALA increases in non-blood tissues likely begin to occur at roughly the same blood lead levels associated with increases in erythrocyte ALA levels.

Lead also affects heme synthesis beyond metabolic steps involving ALA, leading to the accumulation of porphyrin in erythrocytes as the result of impaired iron insertion into the porphyrin moiety to form heme. The porphyrin acquires a zinc ion in lieu of the native iron, and the resulting accumulation of blood zinc protoporphyrin (ZPP) tightly bound to erythrocytes for their entire life (120 days) represents a commonly employed index of lead exposure for medical screening purposes. The threshold for elevation of erythrocyte protoporphyrin (EP) levels is well-established as being 25-30 μg/dl in adults and approximately 15 μg/dl for young children, with significant EP elevations (>1-2 standard deviations above reference normal EP mean levels) occurring in 50 percent of all children studied as blood lead approaches or moderately exceeds 30 μg/dl.

Medically, small increases in EP levels were previously not viewed as being of great concern at initial detection levels around 15-20 μg/dl in children. However, EP increases become more worrisome when markedly greater, significant elevations occur as blood lead levels reach 20 to 30 μg/dl and additional signs of significantly deranged heme synthesis begin to appear, along with indications of functional disruption of various organ systems. Previously, such other signs of significant organ system functional disruptions had only been credibly detected at blood lead levels distinctly in excess of 30 μg/dl, e.g., inhibition of hemoglobin synthesis starting at 40 μg/dl and significant nervous system effects at 50-60 μg/dl. This served as a basis for CDC's 1978 statement establishing 30 μg/dl blood lead as a criteria level for undue lead exposure for young children. At the present time, however, the medical community (CDC, 1985) accepts EP elevations associated with PbB levels of 25 μg/dl or higher as being unacceptable in pediatric populations.

Recently, it has also been demonstrated in children that lead is negatively correlated with circulating levels of the vitamin D hormone, 1,25-dihydroxyvitamin D, with the negative association existing down to 12 μg/dl of blood lead. This effect of lead is of considerable significance on two counts: (1) altered levels of 1,25-$(OH)_2$-vitamin D not only impact calcium homeostasis (affecting mineral metabolism, calcium as a second messenger, and calcium as a mediator of cyclic nucleotide metabolism) but also likely impact its known role in immunoregulation and mediation of tumorigenesis; and (2) the effect of lead on 1,25-$(OH)_2$-vitamin D is a particularly robust one, with blood lead levels of 30-50 μg/dl resulting in decreases in the hormone that overlap comparable degrees of decrease seen in severe kidney injury or certain genetic diseases.

Erythrocyte Py-5-N activity in children has also been demonstrated to be negatively impacted by lead at exposures resulting in blood lead levels markedly below 30 μg/dl (i.e., to levels below 5 μg/dl with no evident threshold). Extensive reserve capacity exists for this blood enzyme, such that it is not markedly depleted until blood lead levels reach approximately 30-40 μg/dl, arguing for the Py-5-N effect in and of itself as perhaps not being particularly adverse until such blood lead levels are reached. However, the observation of Py-5-N inhibition is more arguably indicative of more widespread impacts on pyrimidine metabolism in general in additional organs and tissues besides blood, such that lead exposures lower than 30 μg/dl resulting in measurable Py-5-N inhibition in erythrocytes may be of greater medical concern when viewed from this broader perspective.

Also adding to the concern about relatively low exposure levels of lead are the results of an expanding array of animal toxicology studies which demonstrate the following: (1) persistence of lead-induced neurobehavioral alterations well into adulthood long after termination of perinatal lead exposure early in development of several mammalian species;

(2) evidence for uptake and retention of lead in neural and non-neuronal elements of the CNS, including long-term persistence in brain tissues after termination of external lead exposure and blood lead levels have returned to "normal"; and (3) evidence from various in vivo and in vitro studies indicating that, at least on a subcellular-molecular level, no threshold may exist for certain neurochemical effects of lead.

Given the above new evidence that is now available, indicative of significant lead effects on nervous system functioning and other important physiological processes as blood lead levels increase above 15-20 µg/dl and reach 20 to 30 µg/dl, the rationale for considering 30 µg/dl as a "maximum safe" blood lead level (as was the case in setting the 1978 EPA lead NAAQS) was called into question and substantial impetus provided for revising the criteria level downward. At this time, it is difficult to identify specifically what blood lead criteria level would be appropriate in view of the existing medical information. Clearly, however, 30 µg/dl does not afford any margin of safety before blood lead levels are reached that are associated with unacceptable risk of notable adverse health effects occurring in some children. This is based on at least two grounds: (1) blood lead levels in the 30-40 µg/dl range are now known to "mask", for some children, markedly elevated chelatable body lead burdens that are comparable to lead burdens seen in other children displaying overt signs and symptoms of lead intoxication and (2) blood lead levels in the 30-40 µg/dl range are also associated with the onset of deleterious effects in several organ systems which are either individually or collectively seen as being adverse. These and other considerations have led the medical community (CDC, 1985) to define 25 µg/dl PbB as a level associated with unacceptable risk for pediatric lead toxicity.

At levels below 25-30 µg/dl, many of the different smaller effects reported as being associated with lead exposure might be argued as separately not being of clear medical significance, although each are indicative of interference by lead with normal physiological processes. On the other hand, the collective impact of all of the observed effects (representing potentially impaired functioning and depleted reserve capacities of many different tissues and organs) can, at some point distinctly below 25-30 µg/dl, be seen as representing an adverse pattern of effects worthy of avoidance. The onset of signs of detectable heme synthesis impairment in many different organ systems at blood lead levels starting around 10-15 µg/dl, along with indications of increasing degrees of pyrimidine metabolism interference and signs of altered nervous system activity, might be viewed as such a point. Or, alternatively, the collective impact of such effects might be argued as becoming sufficiently adverse to warrant avoidance (with a margin of safety) only when the various effects come to represent marked deviations from normal as blood lead levels exceed 20-25 µg/dl.

The frequency of occurrence of various effects among individual affected children at various blood lead levels may have important bearing on the ultimate resolution of the above issue regarding the definition of blood lead levels associated with adverse health effects in pediatric populations. The proportion of children likely affected (i.e., responders) in terms of experiencing particular types of effects at various lead levels is also an important consideration. Some information bearing on this latter point is discussed next.

13.6 DOSE-RESPONSE RELATIONSHIPS FOR LEAD EFFECTS IN HUMAN POPULATIONS

Information summarized in the preceding section dealt with the various biological effects of lead germane to the general population and included comments about the various levels of blood lead observed to be associated with the measurable onset of these effects in various population groups. As indicated above, inhibition of ALA-D activity by lead occurs at virtually all blood lead levels measured in subjects residing in industrialized countries. If any threshold for ALA-D inhibition exists, it lies somewhere below 10 μg/dl blood lead.

Elevation in erythrocyte protoporphyrin for a given blood lead level is greater in children and women than in adult males, children being somewhat more sensitive than women. The threshold for currently detectable EP elevation in terms of blood lead levels for children was estimated at approximately 16-17 μg/dl in the recent studies of Piomelli et al. (1982). In adult males, the corresponding blood lead value is 25-30 μg/dl. Also, statistically significant reduction in hemoglobin production occurs at a lower blood lead level in children (40 μg/dl) than in adults (50 μg/dl).

Coproporphyrin elevation in urine first occurs at a blood lead level of 40 μg/dl and this threshold appears to apply for both children and adults. In addition, it appears that urinary ALA shows a correlation with blood lead levels to below 40 μg/dl, but since there is no clear agreement as to the meaning of elevated ALA-U below 40 μg/dl, this value is taken as the threshold for pronounced excretion of ALA into urine. This value appears to apply to both children and adults. Whether this blood lead level represents a threshold for the potential neurotoxicity of circulating ALA cannot now be stated and requires further study.

A number of investigators have attempted to quantify more precisely dose-population response relationships for some of the above lead effects in human populations. That is, they have attempted to define the proportion of a population exhibiting a particular effect at a given blood lead level. To date, such efforts at defining dose-response relationships for lead effects have been mainly limited to the following effects of lead on heme biosynthesis: inhibition of ALA-D activity; elevation of EP; and urinary excretion of ALA.

Dose-population response relationships for EP in children have been analyzed in detail by Piomelli et al. (1982) and the corresponding plot at two levels of elevation (>1 S.D., >2 S.D.) is shown in Figure 13-5 using probit analysis. It can be seen that blood lead levels in half of the children showing EP elevations at >1 and 2 S.D.'s closely bracket the blood lead level taken as the high end of "normal" (i.e., 30 µg/dl). Dose-response curves for adult men and women as well as children prepared by Roels et al. (1976) are set forth in Figure 13-6. In Figure 13-6, it may be seen that the dose-response for children remains greater across the blood lead range studied, followed by women, then adult males.

Figure 13-7 presents dose-population response data for urinary ALA exceeding two levels (at mean + 1 S.D. and mean + 2 S.D.), as calculated by EPA from the data of Azar et al. (1975). The percentages of the study populations exceeding the corresponding cut-off levels as calculated by EPA for the Azar data are set forth in Table 13-9. It should be noted that the measurement of ALA in the Azar et al. study did not account for aminoacetone, which may influence the results observed at the lowest blood lead levels.

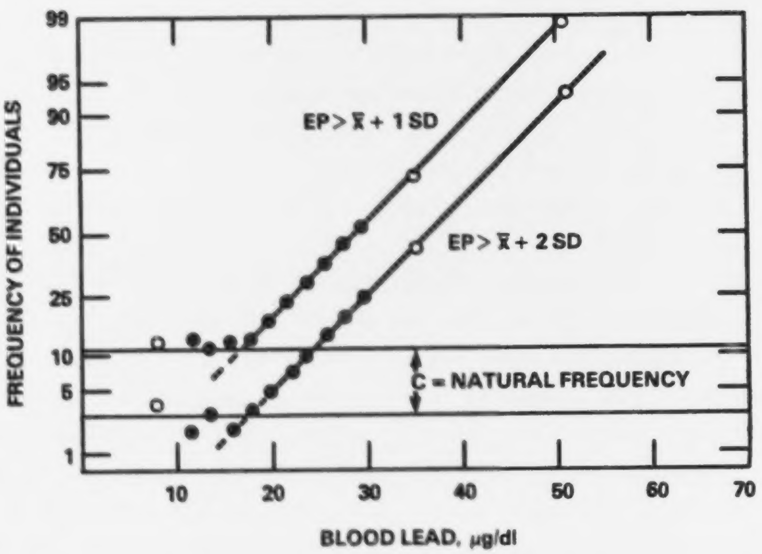

Figure 13-5. Dose-response for elevation of EP as a function of blood lead level using probit analysis. Geometric mean plus 1 S.D. = 33 µg/dl; geometric mean plus 2 S.D. = 53 µg/dl.

Source: Piomelli et al. (1982).

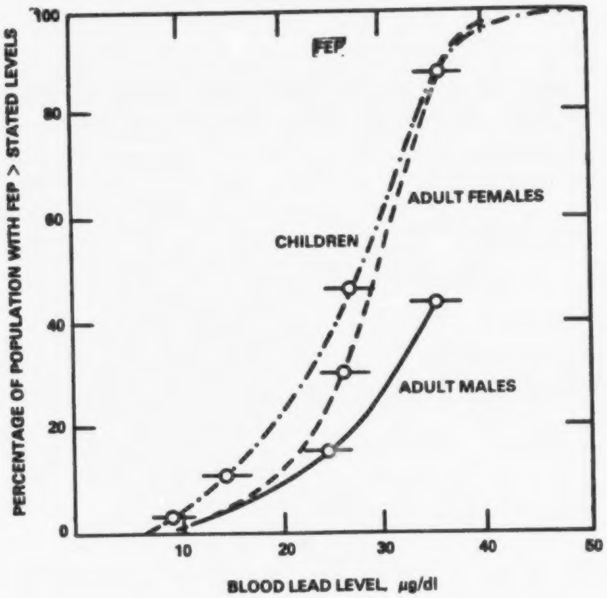

Figure 13-6. Dose-response curve for FEP as a function of blood lead level: in subpopulations.

Source: Roels et al. (1976).

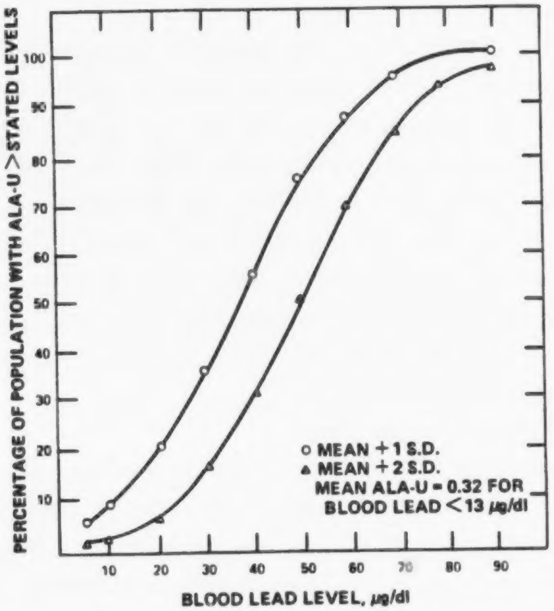

Figure 13-7. EPA-calculated dose-response curve for ALA-U.

Source: Azar et al. (1975).

TABLE 13-9. EPA-ESTIMATED PERCENTAGE OF SUBJECTS WITH ALA-U EXCEEDING LIMITS FOR VARIOUS BLOOD LEAD LEVELS

Blood lead levels, µg/dl	Azar et al. (1975), percent population
10	2
20	6
30	16
40	31
50	50
60	69
70	84

13.7 POPULATIONS AT RISK

A population at risk is a segment of a defined population exhibiting characteristics associated with significantly higher probability of developing a condition, illness, or other abnormal status. This high risk may result from either (1) greater inherent susceptibility or (2) from exposure situations peculiar to that group. What is meant by inherent susceptibility is a host characteristic or status that predisposes the host to a greater risk of heightened response to an external stimulus or agent.

In regard to lead, three such populations are definable: they are preschool age children (≦6 years old), especially those living in urban settings, pregnant women, and white males aged 40-59, although the evidence concerning this latter group is much more limited than that for the other two. Children are such a population for both of the reasons stated above, whereas pregnant women are at risk primarily due to the inherent susceptibility of the conceptus. Also, for reasons not as yet fully understood, the limited information available indicates that middle-aged white males appear to be differentially more at risk for manifesting elevations in blood pressure in response to lead exposure (see the Addendum to this document for a complete discussion of the evidence supporting this).

13.7.1 Children as a Population at Risk

Children are developing and growing organisms exhibiting certain differences from adults in terms of basic physiologic mechanisms, capability of coping with physiologic stress, and their relative metabolism of lead. Also, the behavior of children frequently places them in different relationship to sources of lead in the environment, thereby enhancing the opportunity for them to absorb lead. Furthermore, the occurrence of excessive exposure often is not realized until serious harm is done. Young children do not readily communicate a medical history of lead exposure, the early signs of such being common to so many other disease states that lead is frequently not recognized early on as a possible etiological factor contributing to the manifestation of other symptoms.

13.7.1.1 Inherent Susceptibility of the Young. Discussion of the physiological vulnerability of the young must address two discrete areas. Not only should the basic physiological differences be considered that one would expect to predispose children to a heightened vulnerability to lead, but also the actual clinical evidence must be considered that shows such vulnerability does indeed exist.

In Chapter 10 and Section 13.2 above, differences in relative exposure to lead and body handling of lead for children versus adults were pinpointed throughout the text. The significant elements of difference include the following: (1) greater intake of lead by infants and young children into the respiratory and gastrointestinal (GI) tracts on a body weight basis compared to adults; (2) greater absorption and retention rates of lead in children; (3) much greater prevalence of nutrient deficiency in the case of nutrients which affect lead absorption rates from the GI tract; (4) differences in certain habits, i.e., normal hand-to-mouth activity as well as pica, resulting in the transfer of lead-contaminated dust and dirt to the GI tract; (5) differences in the efficiency of lead sequestration in the bones of children, such that not only is less of the body burden of lead in bone at any given time, but the amount present may be relatively more labile. Additional information discussed in Chapter 12 suggests that the blood-brain barrier in children is less developed, posing the risk for greater entry of lead into the nervous system.

Hematological and neurological effects in children have been demonstrated to have lower thresholds in terms of blood lead levels than in adults. Similarly, reduced hemoglobin production and EP accumulation occur at relatively lower exposure levels in children than in adults, as indexed by blood lead thresholds. With reference to neurologic effects, the onset of encephalopathy and other injury to the nervous system appears to vary both regarding likely lower thresholds in children for some effects and in the typical pattern of neurologic effects presented, e.g., in encephalopathy or other CNS deficits being more common in children versus peripheral neuropathy being more often seen in adults. Not only are the effects more acute in children than in adults, but the neurologic sequelae are also usually much more severe in children.

13.7.1.2 Exposure Consideration. The dietary habits of children as well as the diets themselves differ markedly from adults and, as a result, place children in a different relationship to several sources of lead. The dominance of canned milk and processed baby food in the diet of many young children is an important factor in assessing their exposure to lead, since both those foodstuffs have been shown to contain higher amounts of lead than components of the adult diet. The importance of these lead sources is not their relationship to airborne lead directly but, rather, their role in providing a higher baseline lead burden to which the airborne contribution is added.

Children ordinarily undergo a stage of development in which they exhibit normal mouthing behavior, as manifested, for example, in the form of thumbsucking. At this time they are at risk for picking up lead-contaminated soil and dust on their hands and hence into their mouths where it can be absorbed.

There is, however, an abnormal extension of mouthing behavior, called pica, which occurs in some children. Although diagnosis of this is difficult, children who exhibit this trait have been shown to purposefully eat nonfood items. Much of the lead poisoning due to lead-based paint is known to occur because children actively ingest chips of leaded paint.

13.7.2 Pregnant Women and the Conceptus as a Population at Risk

There are some rather inconculsive data indicating that women may in general be at somewhat higher risk to lead than men. However, pregnant women and their concepti as a subgroup are demonstrably at higher risk. It should be noted that, in fact, it really is not the pregnant woman *per se* who is at greatest risk but, rather, the unborn child she is carrying. Because of obstetric complications, however, the mother herself can also be at somewhat greater risk at the time of delivery of her child. With reference to maternal complication at delivery, information in the literature suggests that the incidence of preterm delivery and premature membrane rupture relates to maternal blood lead level. Further study of this relationship as well as studies relating to discrete health effects in the newborn are needed.

Vulnerability of the developing fetus to lead exposure arising from transplacental transfer of maternal lead was discussed in Chapter 10. This process starts at the end of the first trimester. Umbilical cord blood studies involving mother-infant pairs have repeatedly shown a correlation between maternal and fetal blood lead levels.

Further suggestive evidence, cited in Chapter 12, has been advanced for prenatal lead exposures of fetuses, possibly leading to later higher instances of postnatal mental retardation among the affected offspring. The available data are insufficient to state with any certainty that such effects occur or to determine with any precision what levels of lead exposure might be required prior to or during pregnancy in order to produce such effects.

Studies have demonstrated that women in general, like children, tend to show a heightened response of erythorcyte protoporphyrin levels upon exposure to lead. The exact reason for this heightened response is not known but may relate to endocrine differences between men and women.

13.7.3 Middle-Aged White Males (Aged 40-59) as a Population at Risk

Recently-emerging epidemiological evidence indicates that increased blood pressure is associated with blood lead concentrations ranging from ≥30-40 µg/dl down to blood lead levels possibly as low as 7 µg/dl. This relationship appears to be particularly significant for middle-aged white males (aged 40-59), although a considerable degree of uncertainty surrounds the statistical analyses of the studies giving rise to this conclusion. A detailed critique of the various analyses which have been performed on the available epidemiological studies concerning the blood lead/blood pressure relationship, as well as a discussion of the plausible biological mechanisms underlying this relationship, are presented in Section 1 of the Addendum to this document.

The specific magnitudes of risk obtained for serious cardiovascular outcomes in relation to lead exposure, estimated on the basis of lead-induced blood pressure increase, depends crucially upon the size of the coefficients estimated for the blood lead/blood pressure association. Given the fact that significant uncertainty exists in regard to the most appropriate blood-lead blood-pressure coefficient(s) to use in attempting to project serious cardiovascular outcomes, the further analysis of additional large-scale epidemiological data sets will be necessary in order to resolve more precisely the quantitative relationship(s) between blood lead and blood pressure. It is possible, however, to identify at this time the population subgroup of middle-aged white males (aged 40-59) as being yet another group at general risk in terms of manifesting notable health effects in response to lead exposure.

13.7.4 Description of the United States Population in Relation to Potential Lead Exposure Risk

In this section, estimates are provided of the number of individuals in those segments of the population which have been defined as being potentially at greatest risk for lead exposures. These segments include preschool children (up to 6 years of age), especially those living in urban settings, women of child-bearing age (defined here as ages 15-44), and white males, aged 40-59. These data, which are presented in Table 13-10, were obtained from a provisional report by the U.S. Bureau of the Census (1984). Data from the 1980 Census (U.S. Bureau of the Census, 1983) indicates that approximately 61 percent of the populace lives in urban areas (defined as central cities and urban fringe). Assuming that the 61 percent estimate for total urban residents applies equally to children of preschool age, then approximately 15,495,000 children of the total listed in Table 13-10 would be expected to be at greater risk by virtue of higher lead exposures generally associated with their living in urban versus non-urban settings. (NOTE: The age distribution of the percentage of urban residents may vary between SMSA's.)

TABLE 13-10. PROVISIONAL ESTIMATE OF THE NUMBER OF INDIVIDUALS IN URBAN AND RURAL POPULATION SEGMENTS AT GREATEST POTENTIAL RISK TO LEAD EXPOSURE

Population segment	Actual age, (yr)	Total number in U.S. population (1984)	Urban population*
Preschool children	0-4	18,453,000	11,256,000
	5	3,576,000	2,181,000
	6	3,374,000	2,058,000
Total		25,403,000	15,495,000
Women of child-bearing age	15-19	9,019,000	5,502,000
	20-24	10,481,000	6,393,000
	25-29	10,869,000	6,630,000
	30-34	10,014,000	6,109,000
	35-39	9,040,000	5,514,000
	40-44	7,179,000	4,379,000
Total		56,602,000	34,527,000
White males	40-44	6,064,000	3,699,000
	45-49	4,960,000	3,026,000
	50-54	4,600,000	2,806,000
	55-59	4,760,000	2,904,000
Total		20,384,000	12,435,000

*An urban/total ratio of 0.61 was used for all age groups. "Urban" includes central city and urban fringe populations (U.S. Bureau of the Census, 1983).

Source: U.S. Bureau of the Census (1984), Table 6.

The risk encountered with exposure to lead may be compounded by nutritional deficits (see Chapter 10). The most commonly seen of these is iron deficiency, especially in young children less than 5 years of age (Mahaffey and Michaelson, 1980). Data available from the National Center for Health Statistics for 1976-1980 (Fulwood et al., 1982) indicate that from 8 to 22 percent of children aged 3-5 may exhibit iron deficiency, depending upon whether this condition is defined as serum iron concentration (<40 µg/dl) or as transferrin saturation (<16 percent), respectively. Hence, of the 22,029,000 children ≤5 years of age (Table 13-10), as many as 4,846,000 would be expected to be at increased risk, depending on their exposure to lead, due to iron deficiency.

As pointed out in Section 13.7.2, the risk to pregnant women is mainly due to risk to the conceptus. By dividing the total number of women of child-bearing age in 1981 (56,602,000) into the total number of live births in 1984 (3,697,000; National Center for Health Statistics, 1985), it may be seen that approximately 7 percent of this segment of the population may be at increased risk at any given time.

As for white males, aged 40-59, defined as being at risk most notably for increased blood pressure in association with elevated blood lead levels, approximately 20 million individuals can be estimated to be at potential risk based on the 1980 U.S. Census data.

13.8 SUMMARY AND CONCLUSIONS

Among the most significant pieces of information and conclusions that emerge from the present human health risk evaluation are the following:

(1) Anthropogenic activity has led to vast increases of lead input into those environmental compartments which serve as media (e.g., air, water, food, dust, and soil, etc.) by which significant human exposure to lead occurs. Current blood lead concentrations of populations in industrialized societies best reflect this impact of man's activities, with such lead levels being much higher than those found in contemporary populations remote from industrial activities.

(2) Emission of lead into the atmosphere, especially through leaded gasoline combustion, is of major significance in terms of both the movement of lead to other environmental compartments and the relative impact of such emissions on the internal lead burdens in industrialized human populations. By means of both mathematical modeling of available clinical/epidemiological data by EPA and the isotopic tracing of lead from gasoline to the atmosphere to human blood of exposed populations, the atmospheric lead contribution to human blood lead levels in industrialized areas is estimated to be approximately 25-50 percent.

(3) Given this magnitude of relative contribution to human external and internal exposure, decreases in atmospheric lead levels would then result in significant widespread reductions in levels of lead in human blood (an outcome supported by careful analysis of the NHANES II data). Reduction of lead in food (added in the course of harvesting, transport, and processing) is also be expected to produce significant widespread reductions in human blood lead levels in the United States, as would efforts to decrease the numbers of American children residing in housing with interior or exterior lead-based paint.

(4) A number of adverse effects in humans and other species are clearly associated with lead exposure and, from an historical perspective, the observed "thresholds" for these various effects (particularly neurological and heme biosynthesis effects) continue to decline as more sophisticated experimental and clinical measures are employed to detect more subtle, but still significant effects. These include significant alterations in normal physiological functions at blood lead levels markedly below the currently accepted 25 µg/dl "maximum safe level" for pediatric exposures.

(5) Preceding chapters of this document demonstrate that young children are at greatest risk for experiencing lead-induced health effects, particularly in the urbanized, low-income segments of this pediatric population. A second group at increased risk is pregnant women, because of exposure of the fetus to lead in the absence of any effective biological (e.g., placental) barrier during gestation. A third group at increased risk would appear to be white males, aged 40-59, in that blood pressure elevations appear to be significantly correlated with elevations in blood lead level in this group.

(6) Dose-population response information for heme synthesis effects, coupled with information from various blood lead surveys, e.g., the NHANES II study, indicate that large numbers of American children (especially low-income, urban dwellers) have blood lead levels sufficiently high (in excess of 15-20 µg/dl) that they are clearly at risk for deranged heme synthesis and, possibly, other health effects of growing concern as lead's role as a general systemic toxicant becomes more completely delineated.

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